THE OHIO JOURNAL OF SCIENCE

VOL. XLVIII MAY, 1948 No. 3

SOME PECULIARITIES OF THE MALE FROG TEST FOR EARLY PREGNANCY

D. F. MILLER AND P. B. WILTBERGER[1]

An article describing the use of the male of the leopard frog (*Rana pipiens*) as a new test animal for early pregnancy was published in *Science* for February 20, 1948 (1). This is a rather radical departure from the widely used effects of the gonadotropic hormones upon the female sex organs of test animals. In this new test we have described a method that has as its chief virtues accuracy, rapidity, sensitivity, and simplicity.

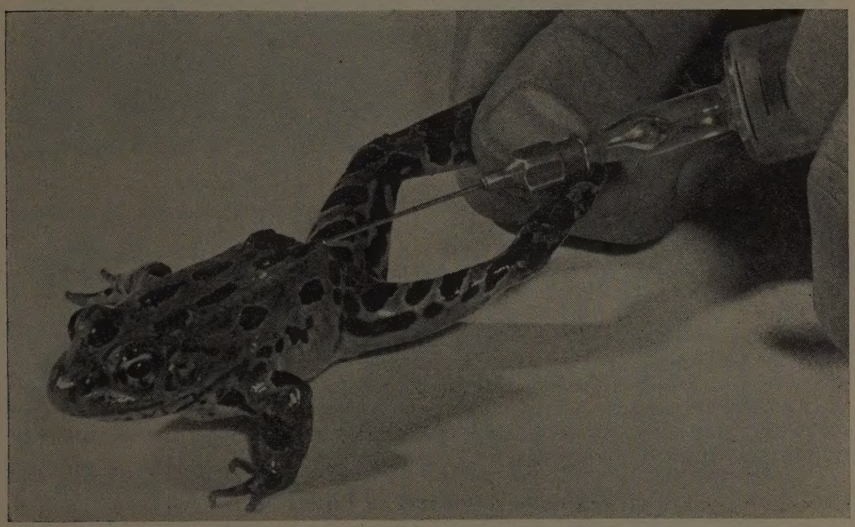


FIG. 1. Method of injecting into the dorsal lymph sac of a frog.

The simplicity of the technic is easily seen from the following description. The patient secures an ounce or more of the first urine she voids upon arising in the morning and sends this to the physician for the test. The technician injects, by hypodermic syringe, from 2 cc. to 4 cc. of the urine into the lymph sacs of a male frog using a 25 gauge needle. Larger needles may allow the urine to leak out. The frog is then placed in a pint fruit jar which has a wide mouth and a perforated lid. The jar is labelled and set aside for a period of from two to four hours during which the frog absorbs the urine. At the end of this period an examination of the

[1]D. F. Miller, Chairman, Department of Zoology and Entomology, The Ohio State University, Columbus, Ohio. P. B. Wiltberger, M.D., 350 East State Street, Columbus, Ohio.

jar will often show the presence of urine voided by the frog. If not, the frog may be made to urinate by seizing its head and shoulders in the hand whereupon the frog will reflexively eject a jet of urine. A drop of the voided urine is placed upon a microscope slide covered with a cover-glass and examined under 16 mm. and 4 mm. objectives. Since the spermatozoa of *Rana pipiens* are large the technician soon learns to recognize them with the 16 mm. lense alone. The presence of the frogs sex cells in the urine indicates a positive result of the test for the presence of the gonadotropic hormone usually found in the blood plasma of pregnant women.

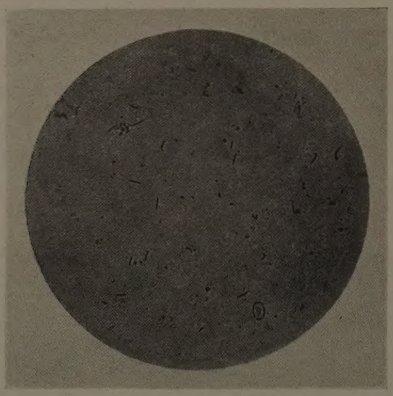


FIGURE 2

FIGURE 3

FIG. 2. Photomicrograph of a drop of frog urine containing frog sperm cells, as seen when using 10× ocular and 16 mm. objective.

FIG. 3. Photomicrograph showing frog sperm cells as seen when using 10× ocular and 4 mm. objective. Cigar-shaped bodies are sperm heads each of which bears a transparent flagellum at one end. There are eight sperms appearing in this illustration.

The absence of the frog sperm cells is a negative result of the test for the hormone. Since the activating agent *seems* to be chorionic in origin its presence in the urine of the women should ordinarilly indicate pregnancy. R. G. Hoskins (2) says that the placenta "apparently also functions as an endocrine gland producing important regulatory hormones. Concentration of the urine insures greater certainty of the results and the use of fewer frogs.

CARE AND HANDLING OF THE FROGS

The leopard (or grass) frog is widely distributed and may be bought from frog farms, biological supply houses, or collected locally. The ideal time to collect them seems to be in early autumn before cold weather has brought on hibernation The testes of such frogs contain mature sperms. They may be kept best in a cold storage room in which the temperature never gets below freezing, from 34° to 40°F. They should be placed in a box of damp peat moss and sprinkled lightly whenever they seem to be getting dry. If a few frogs are brought out of this storage one or two days before they are used in tests they can be kept in a wet place until used. Frogs should be wet at all times but *not kept in water from which they cannot crawl out.* In deep water a frog will drown. A very satisfactory container is a pan or tray 4 inches deep by 18 inches wide by 30 inches long. It should be covered with a lid of half-inch mesh woven wire so hinged that each end of the lid may be opened without opening the whole top of the pan. Such a pan will hold up to 100 frogs

to start. If the pan is tilted so that water will stand in one end while the other is free from water the frogs can keep wet by getting into the water. If the water is not running it should be changed every day. Frogs that are cool will keep for weeks without feeding. Small crayfish, earthworms or mealworms can be fed if the frogs are not in storage, but this is hardly necessary.

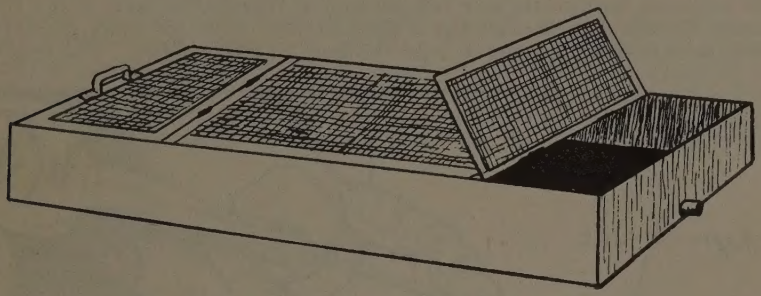


FIG. 4. Tray for keeping a stock of 100 or less frogs.

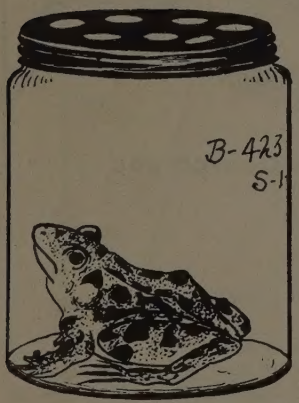


FIGURE 5

FIGURE 6

FIG. 5. Wide-mouthed pint fruit jar with perforated metal lid for holding individual frogs during test.

FIG. 6. Male frogs are distinguished from females by swollen base of "thumb."

If a frog has been used in a test and the test was negative, that frog may be returned to the original pan and used over again at any time. But *if the test was positive* the frog should be placed in another container for at least four days before being reused. Hence two pans should be provided as containers. For the individual physician who wishes merely to run an occasional test for a patient it is possible to have half a dozen frogs which can be kept in a large jar with a heavy screen top. A very thin layer of water in the bottom of the jar is sufficient and this should be changed daily.

In this species of frog (*Rana pipiens*) the sexes are easily distinguished by the difference in the appearance of the base of the first digit (thumb) on the foreleg. It is generally swollen and more heavily pigmented in the male as shown in figure 6. The body of a mature frog of this species should be from two and a half to three

and a half inches in length. Orders to supply houses or collectors should specify "males only", and even then the technician should *carefully check each frog* when it is selected for a test.

The skin of the frog is very loosely attached to the body wall, being fastened by strips of connective tissues which divide the space between the skin and the muscles into large pockets, or lymph sacs. In making an injection the needle should be thrust through the skin but not into or through the body wall. Interperitoneal injection can be made but is likely to result in severe injury to the frog. During the injection it is best to hold the frog by its hind feet. The frog will

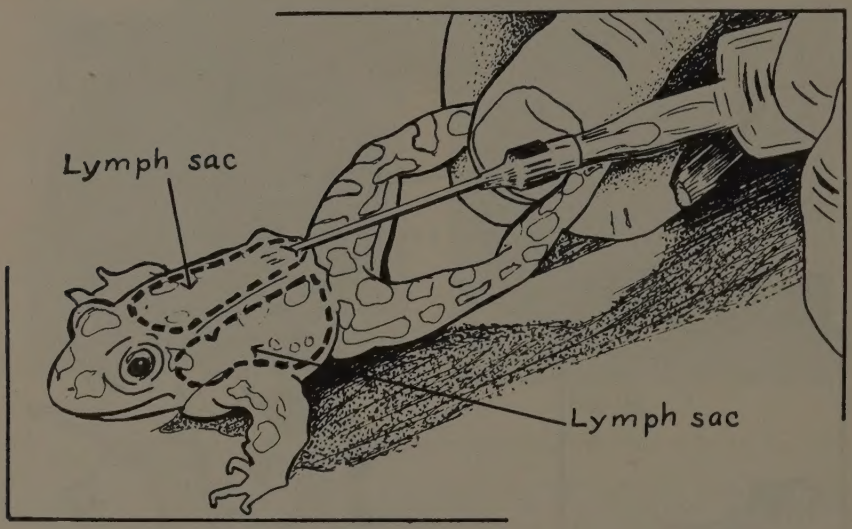


FIG. 7. Showing dorsal and lateral lymph sacs.

straighten out on the table top and does not struggle much during injection. A check on each frog can be made by placing it in a jar for an hour before it is injected. At the end of this time its urine can be examined for sperm cells. It must be negative. Then the frog may be injected for the test, each frog furnishing its own check. We have always recommended that two frogs be run for each test as a precaution against individual differences or in the case where the potency of the hormone is low.

SOME PECULIARITIES AND RESULTS OF THE TESTS

Samples of pregnancy urine may be kept and used for demonstration purposes or for producing live spermatazoa for classroom study. One sample of such urine was kept in the refrigerator for five weeks and then gave a good positive test. The same sample after standing at room temperature for six more days still gave as good a test when fresh. Another sample was kept for several days at room temperature and then preserved by adding two drops of toluene and shaking thoroughly. After standing at room temperature for eight weeks it produced a strong positive test. This seems to indicate that the *gametokinetic substance* does not deteriorate readily. However, some of the commercially prepared anterior petuitary-like substances degenerate rapidly when opened to the air. The commercially prepared substance will give a heavy discharge of sperm cells if they are

used in fresh condition. A sample of urine known to give a strong positive test can be kept and used upon a male frog at any time that living sperm cells are needed for teaching purposes. These large sperms make excellent demonstration material and may be obtained so much more readily than from mammals. Being fresh they are better teaching materials than a stained smear which is so often used. Urine for this purpose cannot be preserved by heating as heating to the boiling point destroys the effectiveness of the urine.

In a few cases we have found that a urine sample is very toxic to the frogs causing death too early to get a test. In these it has been possible to get a successful test by gradual dilutions of the urine and by watching the test animals so as to collect a urine specimen from them before death. As small a sample as 0.5 cc. of pregnancy urine diluted to 2.0 cc. with tap water has been successful in getting a positive test in some such instances. Adjusting the pH to neutrality is also helpful.

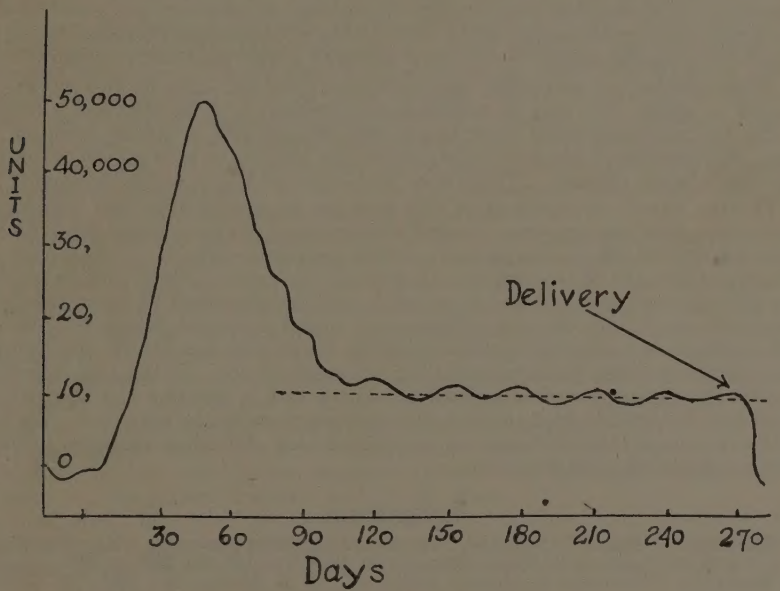


FIG. 8. A composite graph of the abundance of gametokinetic substance in urines during pregnancy. Not taken from any particular study.

In one case of uncertain pregnancy we obtained a urine sample and blood sample at the same time. The urine sample gave a positive test when run with a frog. The blood serum was used upon another frog in the same manner as urine and produced a similar positive test. In another case a women who had recently missed a menstrual period was being given a Friedman test to determine whether she was pregnant. We secured a sample of urine and ran a frog test simultaneously. The frog test was positive, the Friedman test was negative. Ten days later a second Friedman test gave a positive result. Parallel series are being run in several laboratories now.

During our very early studies we expected to accumulate a large number of positive tests in a short time by testing known late pregnancies. We ran 32 tests

in one day and found to our dismay that half of them gave negative results. This looked as if the test was not accurate and we were about to discontinue it when we recalled that studies on blood of pregnant mares and women had shown that the gonadotropic hormone increases very rapidly from conception to 30 days, decreases rapidly up to 90 days, and then continues at a low level around the threshold until delivery, at which time it seems to disappear. All of our tests made during the first trimester of pregnancy had given positive tests. This fact correlated with the abundance of the hormone in the blood during the first 90 days. During the third trimester the level of the hormone was so low that it might be above or below the threshold of sensitivity of the frog. We have tested a few cases during late pregnancy of women whose cycle was regular and who had accurate knowledge of dates and we found that the urine sample taken at the time when menstruation would have occurred gave very weak positive or even negative tests; whereas urine samples taken from the same women between menstrual dates gave strong positive results. This explained our 50 per cent false negatives in the last trimester. It seems that even though the woman is pregnant and bleeding does not occur, there is a lessening of the effect of the gonadotropic hormone during the period when menstruation would have occurred if she were not pregnant.

When testing an extremely early pregnancy, as ten days to two weeks, the number of sperm cells may be very few as compared with 30 days and a recheck should be made one week later which will usually give a strong reaction. It must be remembered, however, that the smaller number of cells is just as positive a test as a larger number. There is no such thing as being *slightly* pregnant.

Further clincal applications of this test are its use in abortions and missed abortions. Our first case was a natural miscarriage. A urine sample taken 6 hours after delivery of the foetus proved positive *indicating that the placenta was still adhering to the wall* of the uterus. Dilation of the cervix proved this to be true. The placenta was removed and a test of a urine sample taken 18 hours later gave a negative result. In the case of suspected missed abortions, where the placenta and uterine wall separate but delivery of the foetus does not follow, the frog test can indicate whether the separation has occurred or not. If the test is positive the placenta is still attached and alive. If the test is negative the placenta has separated, the child is dead and the undischarged mass can be removed. We hope to report more detailed information soon concerning clinical applications in therapeutic and pathological situations.[1]

REFERENCES

(1) **Wiltberger, P. B.** and **D. F. Miller,** "The Male Frog, *Rana pipiens*, as a New Test Animal for Early Pregnancy." *Science*, February 20, 1948, Vol. 107, No. 2773, p. 198.
(2) **MacLeod's** "Physiology in Modern Medicine." C. V. Mosley Co., 1941, Ch. LXXVI; p. 1051.

---

[1]Since this paper was prepared the following article has been published on some clinical applications of the Asheim-Zondek test:

ZONDEK, BERNHARD; FELIX SULMAN, AND RYVKA BLACK. "Hormonal Test for Foetal Death." *Jour. A. M. A.*, Vol. 136, No. 15, p. 965, Apr. 10, 1948.

PETROLEUM GEOLOGY IN THE UNITED STATES GEOLOGICAL SURVEY[1]

HUGH D. MISER
U. S. Geological Survey, Washington 25, D. C.

The drilling of wells in the United States for natural gas began in 1821 in New York and for crude petroleum in 1859 in Pennsylvania. Oil-well drilling spread rapidly over Pennsylvania, West Virginia, Ohio, New York, and Ontario after 1859. Large-scale commercial production and marketing of natural gas began in 1878 when the country's first big gas well was completed in Pennsylvania; and they spread from that State to Ohio and West Virginia in 1884, Illinois in 1885, and Indiana in 1886.

These areas that saw the first commercial production of oil and gas were also to witness the birth and growth of petroleum geology. The early development of petroleum geology was modest and at times irregular, but full stature was attained by it late in the nineteenth century through the contributions of Edward Orton of Ohio and I. C. White of West Virginia. The potentialities of this mature youth were not fully appreciated until 1915 by its parent, the petroleum industry. At that time it was put to work, with all its energy, to guide exploration for new oil and gas fields. The incentive for this full utilization of petroleum geology was the growing demand, early in the twentieth century, of automobile owners, for gasoline to run the mounting number of automobiles.

In the history of our Nation and in the lives of our people our petroleum and natural gas have played a leading role. They have contributed to our welfare and industrial progress; they carried our armed forces to victory in World Wars I and II; and they provide assurance for our country's future welfare and security.

The Geological Survey was founded in 1879, 20 years after the drilling of the Drake well. It directed its first attention to the oil and gas resources of the United States in the late eighties and early nineties when it published reports on petroleum and natural gas in Ohio and Indiana. A continued and intensive program of oil and gas work was begun by the Survey about 1900, and an administrative mineral fuels unit was established in 1908. M. R. Campbell, the first Chief of the unit, was followed successively by David White, K. C. Heald, W. T. Thom, Jr., the present speaker, and C. H. Dane.

Campbell, like other pioneer oil geologists, Orton, J. S Newberry, E. B. Andrews, and John A. Bownocker, was an Ohioan. He received his geologic training under Orton in Columbus. Other Ohioans, besides Campbell, to join the U. S. Geological Survey and become leaders include:

C. Willard Hayes, who was Chief Geologist from 1902 to 1911.

W. C. Mendenhall who was in charge of ground water investigations of the United States, 1907–10; Chief of the land classification board, 1910–20; Chief Geologist, 1922–31; and Director. 1931–43.

Another Ohioan in Washington is Miss Pearl F. Zeigen, who has been my faithful secretary for the past 20 years.

The first report on the geology of petroleum to be published by the U. S. Geological Survey was by Edward Orton. This report on "The Trenton limestone as a source of petroleum and inflammable gas in Ohio and Indiana" was published in 1889 in the Survey's Eighth Annual Report. It followed by several years the

[1] Published with the permission of the Director of the U. S. Geological Survey. This paper was presented as one of the Bownocker Lectures at The Ohio State University on January 20, 1948, and as one of the Grant Memorial Lectures at Northwestern University on March 11, 1948.

direction of Orton's interest to the subject of petroleum geology and was one of many reports that he published on the subject. One of these pioneer papers printed in 1890 was the first one on petroleum geology to be published by the Geological Society of America. At that time Orton and Israel C. White, State Geologist of West Virginia were the foremost authorities on the subject. In the discussion of Orton's paper in the Bulletin of the Geological Society of America, W J McGee, of the United States Geological Survey, paid the following tribute to the work of these two men:

"But within the past three years the laws governing the origin, distribution, and pressure of rock gas have become as well known as are the laws governing artesian water supply; so that today the geologist prognosticates rock gas nearly if not quite as definitely and certainly as he prognosticates artesian water; and it is not only just to our associates and to American science to say that this great advance in geologic science was due almost wholly to two of our fellows—to Professor Orton, the author of the communication before us, and to professor White who has already spoken upon it. To these men we are indebted for this unparalleled stride in American geology."

Orton was the first to publish consistent conclusions on the origin and accumulation of petroleum and on the relation of artesian pressure to oil and gas pressures. Also he brought out the fact that the porosity of the Trenton is dependent on the extent of dolomitization, and that this factor largely governs location of the productive areas scattered over the great Cincinnati arch. He also introduced in the United States in 1888 and 1889 the use of structure contours for showing oil field structure. By means of the contours and shading between them he showed the topography of the Trenton limestone in western Ohio and eastern Indiana.

White published in 1885 his epochal statement on the anticlinal theory of oil accumulation and on his practical application of it. Other geologists including Hunt (1861), Rogers (1860), Logan (1844), and Andrews (1861), had observed the association of oil accumulations with anticlinal axes. Their opinions appear to have little influence on oil exploration and they were strongly opposed by J. P. Lesley. So important was White's statement that Orton proclaimed that his applications of the theory mark a new period in our study of the geology of oil and gas.

It was under Hayes' guidance as Chief Geologist of the U. S. Geological Survey that the refinements of detailed stratigraphic and structural studies were first to be applied in a large way, beginning about 1900. Mining engineers and geologists had, of course, applied similar methods to small areas before Hayes introduced them into the National Survey. It remained for him, however, to show that detailed structures could be worked out over large areas without an inordinate expenditure of time and money. It was his success in the locating of oil pools that led to his being called later to assume charge of the geological work of the Mexican Eagle Oil Co. in Mexico.

About 1900 the U. S. Geological Survey began the examination of a number of oil-producing areas in the Appalachian States, particularly Ohio and Pennsylvania, and structure contours were an essential feature. These reports did much to popularize the use of such contours.

The first of these reports to show the detailed structure of an oil-producing sand by means of structure contours was Bulletin 198, "The Berea grit oil sand in the Cadiz quadrangle, Ohio," by W. T. Griswold. This quadrangle was surveyed in 1901 by Griswold, who introduced the plane table for geologic mapping in connection with his mapping of the topography of the quadrangle.

Industry's adoption of geology as a guide to the study of oil and gas deposits in 1915 was based on the reports of the few geologists in company employment and on reports of the U. S. Geological Survey and various State Surveys, notably the Oklahoma and Kansas Surveys and the California State Mining Bureau.

These reports demonstrated clearly, for many areas, the relation of oil and gas accumulations to anticlinal structure. As a consequence, the early part of the present century witnessed the beginning of a vigorous search for anticlines as drilling sites for oil and gas wells. Also, the Geological Survey made a notable contribution to the development of the oil and gas industries through the training of petroleum geologists who subsequently resigned from the Survey between 1908 and 1925 to join large geologic staffs that were being organized by the oil companies. These geologists, who were thus Geological Survey graduates, became a significant proportion of the leaders in a world-wide search for petroleum.

The oil work of the Geological Survey was greatly accelerated during World War I and again in World War II. In World War I the Geological Survey responded to the imperative need for increasing to the utmost the petroleum supply of the United States, and it largely concentrated its investigations of oil fields in the most promising undeveloped territory, such as Wyoming, and the mid-Continent-Texas region, and especially in the Osage Reservation in Oklahoma. The Osage Reservation demanded particular attention at the time for the following reasons: (1) it contained a great acreage of unleased oil lands; (2) the productivity of the developed areas was high and well sustained; (3) anticlines and domes are numerous in the greater part of the area and the developments and tests indicated that most of the structurally favorable folds would yield oil; and (4) the Office of Indian Affairs, which administers the lands held in common by the Osage Indians, was then offering leases of hundreds of quarter sections to openly competitive bidders on advertised dates. Altogether some 45 geologists and instrument men took part in this investigation of the Osage Reservation under the supervision of K. C. Heald. The long list of their names is most impressive for it is a roster of present-day leaders, of whom two, W. B. Emery and E. M. Spieker, now live in Ohio. To these geologists and instrument men, David White, Chief Geologist, paid the following tribute:

"The field work, which included plane-table mapping with telescopic alidade under many hardships in addition to those of the severe winter (of 1917–18) was carried forward with splendid team work and with gallant and indefatigable emulation. In recording his admiration for the esprit and high standard of this War Work done by geologists in professional civil service, the writer believes he expresses the appreciation not only of the Geological Survey but of the operators as well."

The World War II period of acceleration of oil and gas work began in 1943 and is still in progress; it exceeds in magnitude any oil and gas work heretofore undertaken by the Survey. The number of geologists employed in the Fuels Section in recent years has reached a maximum of about 100 and the number of clerks, draftsmen, and other employees has reached a top figure of about 30.

The purposes of the Survey's present oil and gas work do not differ from those that have guided similar work by the Geological Survey at all other times during the past 50 years. On the other hand, as the methods and techniques of industry have changed during the past 50 years, the nature of the Survey's work has changed.

The mapping of local structural features has been stressed less and less by the Geological Survey and greater emphasis has been placed on regional geologic problems, although some effort is necessarily being directed specifically to the acquisition of information essential for the administration of laws relating to the leasing of public lands for oil and gas development.

The type of work that is being emphasized in recent years is regional geology involving both subsurface and surface stratigraphy in large areas, such as basins or similar geologic provinces. Studies are being devoted primarily to the accumulation of data for the preparation of maps and stratigraphic sections showing such features as the thickness and change in facies of oil-producing formations, the margins of producing or possible producing zones, and the relations and extent of lenticular sands. These studies have as their objective the delimitation of

broad areas that are favorable for exploration. The determination of local structural features, whether by surface, subsurface, or geophysical methods, is not being stressed.

In the formulation and prosecution of the present program of regional stratigraphy and structure the counsel of petroleum geologists and also State Geologists was sought. Many hundreds of petroleum geologists, the officials of numerous educational institutions, and about 24 State Geologists have provided suggestions concerning projects, methods of procedure, and other forms of cooperation and assistance. Also, the program of geologic work is being carried out with the active collaboration of the oil and gas industries, many universities, and the State Geological Surveys. This collaboration assures effective prosecution, without duplication of effort, of types of projects that yield useful results.

The present program of work has been decentralized. Nearly all the work is being done outside Washington. The field employees reside and prepare their reports at project offices. The employees in the Washington office are engaged in the administration of the program, and in the review and processing of reports for publication.

The maintenance of the project offices in the States has many advantages. The field geologists are thus located in areas conveniently near sources of information and are in close personal contact with company geologists and the officials of State Geological Surveys and universities. These contacts give opportunity for the exchange of ideas and for mutual understanding among those who are interested in common problems.

Project field headquarters are now located at the following places: New Philadelphia, Ohio; Ann Arbor, Michigan; Tallahassee, Florida; Tuscaloosa, Alabama; Tulsa, Oklahoma; Lawrence, Kansas; Denver, Colorado; Laramie, Wyoming; Billings, Montana; Albuquerque, New Mexico; and Claremont, California.

Other headquarters that have been used at different times in the past four years are Nashville, Tennessee; Oxford, Mississippi; Morgantown, West Virginia; Austin and Fort Worth, Texas; Norman, Oklahoma; Corvallis, Oregon; and Los Angeles, California.

The different parts of the United States where work by the Fuels Section has been under way during the past four years, include California, Oregon, the Rocky Mountain region from New Mexico to Montana, many States in the mid-Continent region from Texas to Michigan, the southeastern States from Mississippi to Florida, and the Appalachian region from Tennessee and Virginia to Ohio and New York. These areas cover a considerable proportion of the parts of the country that produce petroleum and natural gas or are favorable for their production.

Basic geologic data obtained by the Geological Survey in the course of its work are being made available promptly, through publications, to those directly concerned with the drilling of wells and the testing of new areas. The oil and gas investigations are so planned that useful and timely results can be published rapidly. The results are embodied in large measure in a series of preliminary maps and charts on which brief explanatory texts are printed.

The Geological Survey has printed 110 such maps and charts in its current series. More than 77,000 copies have been sold. Also, during the past 4 years, numerous articles and reports have been submitted for publication to trade and scientific journals and cooperating agencies.

Some types of the preliminary reports are on display on the walls of this auditorium. A complete display of our maps and charts, placed end to end and pasted together, would make a sheet of paper 500 feet long.

Petroleum geology in the U. S. Geological Survey is largely centered in the Fuels Section of the Geologic Branch. The work of this section is closely coordinated with the fields of other sections of the Geological Survey that deal primarily with paleontology, stratigraphy, chemistry, physics, geophysics, mineralogy, and

petrology. In fact, much of the work of these several sections, outside their collaboration with the Fuels Section, is devoted to providing basic information that is utilized by the oil and gas industries in their search for, and development of, our oil and gas resources. Thus, much of the geologic effort of the Geological Survey is related to oil and gas, either directly or indirectly.

In addition to the geologic work of the Fuels Section, some studies that are essential for the administration of the oil and gas bearing public lands are conducted by the Conservation Branch of the Survey. Moreover, intensive studies of the oil and gas resources and possibilities of Alaska have been made since 1903 by Geological Survey geologists. E. M. Spieker has participated in pioneer work in recent years in that frontier area.

The Appalachian project with headquarters at New Philadelphia, Ohio, is typical of many regional projects that were begun in 1943 to assist in the search for much needed oil and gas.

In August 1943, James F. Pepper, the chief of the party, and I made a trip through the Appalachian Basin to see what might be the consensus of geologists of the area as to the type of work that would be most useful to industry. We visited the geological surveys of Ohio, Pennsylvania, and West Virginia, and the geological societies at Charleston, West Virginia, and at Pittsburgh, Pennsylvania.

The decisions reached during this canvas were as follows:

1. Because the project was financed by Federal funds the problems should cover several States and be regional in scope.

2. No regional subsurface studies of a producing sand had ever been made in the Appalachian Basin, nor had extensive surface and subsurface studies been made of a sequence of related beds to determine in so far as possible (a) the facies changes over a broad region, (b) the source areas of the material in the beds, (c) the manner of deposition of the sediments, and (d) the paleogeography of the producing sand and related beds.

The conclusion was reached that an intensive study of a sand following the above outline would be most useful. Before a suitable sand for study could be decided upon several other definite requirements had to be met, namely,

1. The sand must be, or have been, a prolific producer of oil and gas.

2. Many hundreds of well records and locations must be readily available in the files of State organizations and private industry.

3. Since personnel would have to be trained on the job, the correlation problems of at least a part of the area must be at a minimum.

The Berea sand seemed most nearly to fulfill all of the requirements and it was selected, therefore, for intensive study.

All of the personnel of the Appalachian Project arrived by October 1943. The party at that time consisted of nine geologists, a clerk, and a draftsman. Most of the geologists were new to oil and gas geology. One geologist of the group, Gordon Rittenhouse, was a specialist in sedimentary petrography. He was assigned to Morgantown, West Virginia, where the State Geological Survey made available an exceedingly well equipped laboratory for his use.

The project quickly lined up into the following procedures:

1. Several geologists were assigned to the field to copy the many well records and locations required in the study.

2. Other geologists in the New Philadelphia office located the wells on topographic maps, plotted the well records and made a correlation of the Berea sand between wells. The thickness of the Berea sand in each well was spotted in its appropriate location and the thicknesses were drawn together by contours or isopachous lines.

The region in Ohio, Pennsylvania, West Virginia, and Kentucky, in which the Berea sand is present, was divided arbitrarily into six nearly equal areas for convenience in collecting and correlating data and for publication of the data.

The completed preliminary study of the Berea sand consisted of six preliminary maps, two of which have since been revised, a preliminary chart (No. 21) by Wallace deWitt, Jr., and a map showing the distribution of several types of Berea sand by Rittenhouse. A map (No. 5) of an older sand, the second Berea sand of Ohio, was published also.

In the study of sand in a large region, such as the Appalachian Basin, much information is acquired. A great part of the information is suitable for the study of sedimentary deposits. For instance, a side issue was a study of the areal extent and thickness of salt beds in Ohio by Pepper, a study of which, if not purely scientific, will be of use to an industry interested in obtaining salt for chemical or other purposes, to say nothing of the hilarity already induced by the coincidental association of names.

Altogether, as a result of the initial investigation of the Berea sand in Ohio, Pennsylvania, West Virginia, and Kentucky, 22 preliminary maps and articles have been published. These publications, as previously mentioned, are an outgrowth of the vast supply of information now on hand. The papers have included grain size analyses, procedural techniques, and an analysis of the paleogeography of the Greenbrier limestone by Rittenhouse.

As a result of the studies of the Berea sand, the information from all preliminary maps and other sources has been compiled into a manuscript for a professional paper. At present, this report is, in its preliminary writing, about 95 per cent complete.

The present plans of the project include an intensive study of the Clinton sand. The compilation of the preliminary Clinton data is now nearly complete and the material is now ready for study. Other work in the Appalachian Basin will include surface mapping of the Dunkirk formation of Devonian age in New York. This study, it is hoped, will determine the correlations and stratigraphic relations of the Dunkirk and Wiscoy formations from Lake Erie eastward to central New York. The results of this study will aid further in the correct interpretation of the stratigraphic relations of the formations in several quadrangles in central New York which, at the present time, are about three-fourths mapped.

It goes without saying that a project covering such a large region (approximately the size of California or of Great Britain) would be futile and its results entirely negligible if it were not for the willing and general cooperation of state surveys, oil and gas companies, and individual operators. One of the unknown factors at the start of the Appalachian Project was the extent to which all who were interested in oil and gas in the Appalachian Basin would cooperate with the Survey. Time has shown that the cooperation, interest, and generosity of all contributors have been exceeded in no other area in the United States. It must be remembered that the Appalachian Basin has no regional scouting service, and with the exception of an agency in Charleston, West Virginia, (specializing in West Virginia well records), no log service of any kind. The generosity of organizations and individuals is all the more remarkable considering the amount of time which they have given the Survey and the amount of trouble members of the Appalachian Project have imposed upon them in obtaining well records. For example, the Appalachian project, in copying well records at the Ohio Fuel Gas Company, totaled a full year's working time for one person. To the Geological Department of the Ohio Fuel Gas Company and to its parent company, the Columbia system, and other subsidiary groups, the Appalachian Project owes a deep debt of gratitude. In Ohio the Appalachian project owes gratitude to the State Geological Survey and its members, past and present,—particularly to Mr. Wilber Stout, ex-State Geologist, to Mr. R. E. Lamborn, Assistant State Geologist, to Dr. George W. White, recent State Geologist, and to Mr. John H. Melvin, the present State Geologist. These companies and individuals and also the Pure Oil Company of Newark, Ohio, and the independent producers of Marietta, Cambridge, and Canton, Ohio,

are only a few of the many in Ohio, Pennsylvania, West Virginia, and Kentucky who have been of great assistance to the project, for without their assistance the record of the Appalachian project would be entirely barren of results.

The United States has for many years produced about three-fifths of the world's petroleum. Continuation of this rapid rate of drawing on our supplies will hasten the approach of the day when the discovery rate of petroleum will decline to the point where our crude petroleum supplies will not meet fully our needs. Adequate future supplies of petroleum products must at all times be assured for the security and welfare of the Nation. Sources of these products are not alone the crude petroleum in this and other countries but also substitute sources which include oil shale, coal, oil-saturated sands, and natural gas. The domestic deposits of these substitutes sources are large, as established by studies of the U. S. Geological Survey, State agencies, and industry, and they are capable of providing ample petroleum products for fully 1,000 years. The Nation must be equipped with fuller and more adequate information than now available concerning the extent, character, and reserves of these substitute sources and concerning processes of large-scale recoveries of petroleum products from them. This is necessary so that they may be utilized promptly when the Nation is required to turn to them to augment domestic and imported supplies of crude petroleum.

A PROPOSED NEW GENUS NAME MARGANANA AND THE ALLOTYPE DESCRIPTION OF PRAIRIANA HIRSUTA DeL.—GYPONINAE

(Homoptera: Cicadellidae)

DWIGHT M. DeLONG
The Ohio State University

The genus name *Margana* was used by Walker in 1865 to designate a group of Lepidoptera in "A List of Lepidopterous Insects of the British Museum. 34, 1520." This same name was used by DeLong to designate a genus of Cicadellidae in the Gyponinae in 1942.[1] A new genus name *Marganana* is thus being proposed for the preoccupied name *Margana*. The type of the genus is *Ponana suilla* Ball.

In the same paper, page 77, the species *Prainiana hirsuta* DeLong was described from two male specimens, one collected at Phoenix and the other at Yuma, Arizona. Recently a large series of males and females was collected at Phoenix, Arizona by Mr Robert A. Flock. The female is therefore being described.

The vertex is a little shorter than male, about three fourths as long as the basal width between the eyes. Length of female, 8.5 mm.

The color of the female is yellowish brown. The elytra of the male are usually dark brown; the elytra of the female are unicolorous with the vertex and pronotum.

The posterior margin of the seventh sternite of the female is deeply, broadly excavated either side of the middle and the laterally produced lateral angles. These excavations form a rather narrow produced lobe at the middle which is exceeded in length by the lateral angles. The female allotype is from Phoenix, Arizona, collected by Robert A. Flock.

---

[1] A Monographic Study of the North American Species of the Subfamily Gyponinae (Homoptera: Cicadellidae). The Ohio State Univ. Press, 1942.

A METHOD FOR REMOVING TRANSMISSIBLE LYSINS FROM SECONDARY CULTURES OF BACTERIA[1]

R. C. THOMAS,

The Ohio Agricultural Experiment Station,

Wooster, Ohio

INTRODUCTION

Precursors of transmissible lysins or bacterial viruses may exist for a given bacterium wherever organic matter is found. The writer has been particularly interested in such sources as seed and grain extracts, crop residues, and the sap of living plants (Thomas 5, 6, 7). Since the addition of grain or plant extracts to primary cultures of bacteria, and also in some instances to secondary cultures, starts the transmissible lysis phenomenon, it seems reasonable to assume that the lytic factor might be removed and the original culture reclaimed if suitable methods could be devised for that purpose.

In a number of instances, bacteria have been rendered apparently free of the lysins associated with them. The method used is outlined in this paper.

It was thought advisable in this preliminary investigation to work only with known cultures. This means that the primary culture and source of the lysin in each instance, were known. The species of bacteria used in this investigation were assumed to be primary species. A comparison of the types of growth of a culture of bacteria, with and without a lysin present, in the same kind of liquid medium furnishes valuable information and enables one to judge from the character of growth of an unknown isolate, whether or not a lytic factor is present. So long as a transmissible lytic factor is in intimate contact with a culture, the secondary growth which develops is no longer susceptible to the same lysin, because usually in a secondary growth, the lytic factor continues to develop with the organism indefinitely. The presence of a lysin can be demonstrated by testing the bacteria-free filtrate of a secondary culture against the primary or original organism. These secondary cultures are usually considered to be resistant to lytic action. Such cultures are so saturated with the lysin that the addition of more has no effect. The lysin-organism relationship of secondary cultures has never been satisfactorily explained.

The possibility of removing a transmissible lysin from a bacterium would depend upon the kind of affinity or relationship which exists between the two—whether the combination is fixed or reversible. A host-parasite relationship might be expected to be irreversible and there could not be much hope of bringing about a separation. On the other hand, if the lytic condition is due to an acquired enzyme or nucleoprotein, a separation may be feasible. In any case, it would be necessary to find an adsorbent which would have at least as great, if not greater, affinity for the lytic substance than its homologous bacterium.

Early investigators of nucleic acids discovered that these acids have a remarkable property of removing proteins from solution. The combination of albumin and syntonin with various nucleic acids was studied by Milroy (4). Evidence seems to indicate that the viruses are at least in part protein. Tobacco mosaic virus is thought to be a nucleoprotein. It was therefore decided to determine what effect nucleic acids might have upon the lytic factor bacterium combination of different species of bacteria and to find out if an organism could be rendered again susceptible to lysis by removal of the virus by adsorption on nucleic acid.

[1]Published with the approval of the Director of the Ohio Agricultural Experiment Station.

METHODS OF INVESTIGATION

Nucleic acids are available commercially from only a few different sources. Preparations from yeast, wheat germs and thymus were obtained. Since these acids are insoluble in water it was necessary to add an alkali to effect solution. For this purpose either sodium hydroxide or sodium bicarbonate solution was used. The preparation from animal tissue was furnished as a sodium salt and was adjusted by the addition of dilute acetic acid pH 6.50 to 7.00.

Other extracts containing nucleic acid from alfalfa, timothy, oats and potato were prepared by digesting 50 grams of the finely ground material with 5 per cent ammonium hydroxide. Extraction was continued for about three hours, with frequent stirring, on a water-bath heated to 70°C. The liquid was then removed by filtering, the ammonia neutralized with hydrochloric acid and the precipitate which formed was collected by centrifuging. The crude extract was redissolved with ammonium hydroxide and reprecipitated twice, then washed to remove ammonium chloride, and finally suspended in 50 milliliters of distilled water. A few drops of sodium hydroxide were added to bring the suspension into solution. These crude extracts were used without further attempts at purification.

A 2 per cent solution of each commercial nucleic acid was prepared. Whatever micro-organisms were present were removed by filtering through a sterile Selas candle. One milliliter of the filtrate was added to 8 milliliters of sterile nutrient broth or distilled water. Then, each tube containing sodium nucleate was inoculated with one drop, approximately $\frac{1}{25}$ of a milliliter, of a secondary culture containing a transmissible lysin. After thoro mixing the preparation was allowed to stand at room temperature for periods varying from 1 to 12 hours. The period of contact of the lytic factors with the different cultures varied from 2 weeks to 3 months. This did not seem to be of much importance. If it was at all possible to free some of the organisms of a culture from the lysin the time of previous contact or association was of little significance.

At varying intervals, after a culture had been in contact with a nucleate, dilution plates were poured, using nutrient agar. These plates were incubated for 3 or 4 days at 28°C. Then single colonies were selected and transfers made to tubes of broth. Dilution plates were made also of the original culture which did not contain a lytic substance. Using the type of colony growth found in these plates as a guide, similar colonies were picked from the plates prepared from the secondary culture treated with sodium nucleate. Usually only surface colonies, with regular or entire margins showing a smooth type of growth, were selected. If the primary culture developed a pigment, then only colonies showing the same shade of color were chosen for transfer.

After incubation for 24 hours a comparison of the growth of the new isolates with that of the parent culture furnished a good indication if any had been selected which were lysin-free. Colonies still containing a lysin either failed to make any growth or developed only a slight clouding of the broth. The isolates which grew normally were next tested against the original virus filtrate. If they showed the same susceptibility to lysis as the primary culture, they were assumed to be virus-free. Cultures of isolates which still retained the virus were not susceptible to lysis by the original lytic factor. The presence of a lytic factor in an isolate which shows only limited growth can be demonstrated readily by testing the filtrate of such a culture against the parent culture or against those isolates from which the factor has been removed. This procedure can be used for analyzing a species of bacteria which may be suspected of harboring a virus, thus rendering it virus-free and of learning the nature of the original form from which the secondary strain was derived.

RESULTS

In table I is shown a number of species of bacteria for which transmissible lysins were developed from known sources and from which they have been removed. The criterion of lysin removal is based on the fact that the new isolates, following treatment with a sodium nucleate, regained susceptibility to the original lysin. So long as a lytic factor was in association with a secondary culture it did not show any evidence of lysis.

In nearly all cases, secondary cultures developed from the bacterial species recorded in table I retained their lysins in symbiotic relationship regardless of how many transfers were made in such media as nutrient broth or agar. One exception was *Xanthomonas pruni* which could be rendered lysin-free by merely plating in nutrient agar. This could not be done with any of the other secondary cultures without previous contact with some kind of nucleic acid or plant extract. All of

TABLE I

NUCLEIC ACIDS AND PLANT EXTRACTS ARE USEFUL AS ADSORBENTS OF TRANSMISSIBLE LYSINS

Organism	Source of lysin	Source of adsorbent	Period of contact with adsorbent	Number of isolates	Number reacting with original lysin
Xanthomonas juglandis	Oat extract	Thymus	9 hrs.	6	2
Xanthomonas stewarti	Oat extract	Wheat germ	12 hrs.	12	10
Erwinia carotovora	Oat extract	Wheat germ	3 hrs.	6	1
Corynebacterium sepedonicum	Potato vine	Potato extract	2 hrs.	18	10
Corynebacterium michiganense	Oat extract	Wheat germ	4 hrs.	6	4
Xanthomonas pruni	Oat extract	Wheat germ	3 hrs.	6	6
Escherichia coli	Animal	Wheat germ	1 hr.	6	5
Escherichic coli	Animal	Wheat germ	2 hrs.	6	5
Escherichia coli	Animal	Wheat germ	7 hrs.	6	0
Staphylococcus aureus	Human	Wheat germ	2,hrs.	4	2
Unidentified species AC	Oat extract	Wheat germ	1½ hrs.	8	7
Unidentified species 1A	Alfalfa extract	Wheat germ	3 hrs.	5	5
Unidentified species 1A	Clover extract	Wheat germ	3 hrs.	5	5
Unidentified species 1A	Timothy extract	Wheat germ	3 hrs.	5	0
Agrobacterium tumefaciens	Oat extract	Thymus	4 hrs.	12	5
Agrobacterium tumefaciens	Oat extract	Timothy	4 hrs.	6	6
Agrobacterium tumefaciens	Oat extract	Alfalfa	4 hrs.	6	6
Agrobacterium tumefaciens	Oat extract	Oat grain	4 hrs.	6	4

the nucleic acid salts and plant extracts used as lysin adsorbants give Feulgen's reaction. Agar-agar also gives this reaction. Probably there is sufficient adsorbing material in agar-agar to remove the lytic factor from *Xanthomonas pruni*.

There does not seem to be much correlation between the source of lytic factors and the kind of nucleic acid used to remove them. Thymus nucleic acid was effective for the lysins of *Xanthomonas juglandis* and *Agrobacterium tumefaciens*, both of which came from a plant source. Nucleic acid from wheat germs removed lytic factors from *Escherichia coli* and *Staphylococcus aureus*, the lysins being of animal origin in both cases.

The only instance of strict specificity is found with *Corynebacterium sepedonicum*. The lysin for the organism had come from an extract of a dead potato vine which had succumbed to the ring-rot disease. An extract of potato tuber only was found to be of value for adsorbing the lysin of that organism and thus restoring it to its primary form and rendering it again susceptible to lysis by the original lytic factor.

The necessary period of contact of a secondary culture with the nucleic acid varied with the organism, and the optimum time could be determined only by trial platings at stated intervals. Those which yielded best results are recorded for each culture in table I. For *Escherichia coli* a contact period of one or two hours before a sample was removed for plating gave virus-free colonies, but 7 hours were too long. Nucleic acids and plant extracts prevent the growth of some species of bacteria indefinitely while other species start to grow sooner or later. Growth of *Escherichia coli*, before the 7-hour period of contact would account for the multiplication of the virus and also for the fact that no colonies could be obtained susceptible to lysis at that time.

Nucleic acids from four different sources were effective for adsorbing lysin from a secondary culture of *Agrobacterium tumefaciens*. When a secondary culture of this organism, without previous contact with a nucleic acid, was plated on nutrient agar, no colonies were ever found free of the lytic factor.

Cultures of virus-free bacteria killed by heating at 60°C. for 30 minutes were used instead of nucleic acid in order to determine if the lytic particles would be fixed on the dead bacteria. No organisms from which the lytic factor had been separated were ever obtained by platings from such preparations.

The removal of a lytic factor from a culture, thus restoring it to susceptibility to lysis and to normal growth in ordinary media, does not furnish assurance that the reclaimed culture is identical with the original in all respects. In order to gain further information in this connection, plant inoculation was tried. Tomato plants were inoculated from cultures of *Agrobacterium tumefaciens* with and without a lysin present and also with isolates from which the lytic factor had been removed and which had again become susceptible to lysis.

The presence of a lytic factor developed from oat grain extract in a culture of *Agr. tumefaciens* prevented that organism from developing galls when inoculated into tomato plants. After the lysin had been removed by treatment with timothy extract, the culture was again rendered susceptible to lysis and also regained capacity to produce galls.

DISCUSSION

Many species of bacteria, particularly primary forms, acquire lytic factors readily when they come into contact with the juices of higher plants, seed extracts and plant residues. The presence of a lytic factor in a culture somehow changes the metabolic activity of the organism. Evidence of this is indicated by failure of the secondary culture to grow in the same medium as the primary, by the need for vitamin or amino acid amendments and by change in color.

With a more definite understanding of the sources of lytic factors it is proper to raise the question regarding any recognized species of bacteria, whether it is a primary or secondary form. This consideration may have a definite bearing upon our viewpoint of classification and nomenclature in the future. If we can take a primary organism and develop a number of different variants or strains with the aid of plant or seed extracts our conception of what constitutes a species or a strain will be brought more definitely into focus.

Secondary forms of bacteria may be anticipated in fermentation processes where the substrate is seeds, grains or plant material which have not been thoroly sterilized by heat.

The fact that nucleic acids are effective in removing transmissible lytic factors from bacteria, thus permitting the organisms to develop as they did before a lysin was added to a culture, suggests that a class of enzymes known as nucleases may enter into lysin formation. A nucleic acid might be expected to have greater affinity for a nuclease than for other enzymes or proteins.

Very little is known regarding the nucleases, particularly their action on living substrates. Ribonuclease was prepared from commercial pancreatin by Dubos and Thompson (1). Kunitz (3), using different methods, isolated the same enzyme in crystalline form from the same source. This particular nuclease readily attacks the sodium salt of yeast nucleic acid. Greenstein (2) reports that thymonucleodepolymerase occurs not only in animal but also in plant tissues. Extracts of the embryos of corn, wheat, pumpkin, sunflower and lima bean exert a strong depolymerizing effect upon sodium thymonucleate. Apparently the distinction between plant and animal nucleic acids is rapidly disappearing. This fact is also evident in the general lack of specificity among nucleic acids in separating lytic factors from bacteria.

SUMMARY

Transmissible lytic factors can be removed from bacteria and the organisms restored to their original form.

Organisms which have been rendered lysin-free again become susceptible to lysis by the original lytic factor. In the case of *Agrobacterium tumefaciens*, the recovery of the capacity to induce gall formation in tomato was demonstrated following treatment with timothy extract.

The method of procedure outlined in this study can be applied to a culture which may be suspected of having a transmissible lysin associated with it. The parent primary culture frequently can be recovered.

LITERATURE CITED

1. **Dubos, Rene J.** and **R. H. S. Thompson.** The decomposition of yeast nucleic acid by a heat-resistant enzyme. Jour. Biol. Chem. 124: 501–510. 1938.
2. **Greenstein, Jesse P.** Ribonuclease and thymonucleodepolymerase. National Cancer Inst. Proc. Part II, Vol. 1: 113. 1942.
3. **Kunitz, M.** Crystalline ribonuclease. Jour. Gen. Physiol. 24: 15–32. 1940.
4. **Milroy, T. H.** Ueber die Eiwiss-Verbingdungen der Nucleinsaure und Thyminsaure und ihre Beziehung zu den Nuclein und Paranuclein. Zeitschrift fur Physiol. Chemie (Hoppe-Seyler's) 22: 307–326. 1896.
5. **Thomas, R. C.** Transmissible lysins in water extracts of seeds. Science 88: 56–57. 1938.
6. ——— Additional facts regarding bacteriophage lytic to *Aplanobacter stewarti*. Phytopath. 30: 602–611. 1940.
7. ——— The bacteriophage reaction for the identification of bacteria. Ohio Agr. Exp. Sta. Tech. Bull. 11, pp. 1–12. 1947.

A SYNOPSIS OF THE GENUS IOWANUS IN NORTH AMERICA INCLUDING MEXICO

DWIGHT M. DeLONG AND RUTH V. HERSHBERGER,
Department of Zoology and Entomology,
The Ohio State University

While discussing the Phlepsids of Mexico and Central America in 1918, Ball[1] erected several new subgenera, one of which he named *Iowanus*. He cited as the genotype *Phlepsius* (*Iowanus*) *handlirschi* Ball, a Mexican species occurring in the state of Guerrero. Other species placed in this subgenus by Ball are *majestus* O. & B., *elongatus* Ball, *hebraeus* Ball and *spatulatus* V. D.

In characterizing this genus Ball states, "Resembling *Phlepsius* but with head much narrower than pronotum, vertex narrow, disc flat, front long and narrow, but slightly widening above. Pronotum large, pushed forward between the eyes, the lateral margins long and strongly oblique. Elytra very long and narrow over one-third their length beyond clavus; central apical cell long, curved, narrow at base. Surface of elytra regularly inscribed with vermiculate lines."

In 1939 the senior author[2] described four species of this genus which are closely related to *majestus* under *Texananus*. A study of this material in comparison with *Texananus* indicates that *Iowanus* should be given generic rank. Ten previously described species are being placed in the genus and two new species are being described and placed in the genus in this synopsis.

KEY TO SPECIES

1	Body narrow, not widened at middle; female segment strongly produced, extending almost to apex of pygofer; sides concavely rounded to narrow sharp pointed apex; male unknown. (Species known only from Mexico)..... **elongatus**
1'	Body broader, definitely broadened at about half its length; female segment either truncate or slightly produced, not nearly as long as pygofer.....2
2(1')	Vertex with a definite black or dark brown transverse band, usually interrupted at middle, between anterior margins of eyes.....3
2'	Vertex with brownish mottling forming an irregular band between anterior margins of eyes.....12
3(2)	Apical half of vertex (portion anterior to black band) white, unmarked; female segment shallowly emarginate either side of a pair of short broad separated median teeth. (Known only from Mexico)..... **apicalis**
3'	Apical half of vertex same color as basal portion, usually with a pair of small approximate spots.....4
4(3')	Vertex bluntly produced, almost half as long at middle as basal width between eyes; female segment roundedly produced from lateral margins to form a broad bifid apical tooth which occupies the median fifth of the posterior margin; male pygofer without caudal spines..... **sabinus**
4'	Vertex more broadened, rounded at apex, scarcely angled; female segment almost truncate, not forming a produced median tooth; male pygofer with caudal spines....5
5(4')	Color rather dark brown; female segment slightly produced at middle with a median rather broad, shallow notch; male aedeagus composed of a single process which is long and slender..... **hebraeus**
5'	Color more chestnut brown; female segment with a deep or narrow median incision or both; male aedeagus composed of an upper and a lower portion.....6

[1]Ball, E. D. Annals Ent. Soc. Amer., 9: 381–389, 1918.
[2]DeLong, D. M. Ohio Jour. Sci., 39: 235–238, 1939.

6(5') Ventral portion of aedeagus cleft for more than half of the apical part forming a pair of long slender processes which are lyre shaped in ventral view.....**lyratus**
6' Ventral portion of aedeagus not cleft or cleft for only a short distance of apical portion..7
7(6') Male styles slender at apical half and tapered to a pointed apex.....**handlirschi**
7' Male styles broader, blunt at apex, aedeagus notched or cleft at apex.....8
8(7') Apex of each apical fork of ventral portion of aedeagus bifid.....**dicentrus**
8' Apex of each apical fork of ventral portion of aedeagus pointed not bifid.....9
9(8') Ventral aedeagus portion sharply bent ventrally and produced for more than one-third its length at apex.....**borrori**
9' Ventral portion of the aedeagus with the apical portion not sharply bent ventrally, or with bent portion very short.....10
10(9') Apical pygofer lobe broadly rounded, as broad as long, constricted at base; dorso-anterior lobe of dorsal portion of aedeagus broadened apically, constricted at base, **bullatus**
10' Apical pygofer lobe elongate or short, not constricted at base; dorso-anterior lobe of dorsal portion of aedeagus narrowed at apex, not constricted basally.....11
11(10') Apical pygofer lobe appearing short in lateral view, bent inwardly, not constricted basally; apical branches of ventral aedeagus slender, elongate, in lateral view appearing bent twice.....**caducus**
11' Apical pygofer lobe elongate, ovate; apical branches of ventral aedeagus short, thick and curved ventrally.....**majestus**
12(2') Male plates elongate, sloping on outer margins to bluntly pointed apices.....**spatulus**
12' Male plates longer, more narrow, the apical half narrow and tapered to slender, acutely pointed apices; pygofer longer.....**ultratus**

Iowanus elongatus (Ball)

Phlepsius (*Iowanus*) *elongatus* Ball, Annals Ent. Soc. Amer. 11: 382, 1918.

Resembling *majestus* but narrower with a long pointed female segment. Length 10 mm.

Vertex angled, about one-third longer at middle than next the eyes, about one-half wider than long. The transverse band covers almost the anterior third of the vertex leaving a pale anterior margin and a median line. Pronotum with three rather distinct dark brown spots near anterior margin behind each eye.

The female last ventral segment tapering from the lateral angles to a long pointed apex which extends almost to the apex of the pygofers.

This species is known from the female holotype specimen only which was collected in Guerrero, Mexico.

Iowanus majestus (Osborn and Ball)

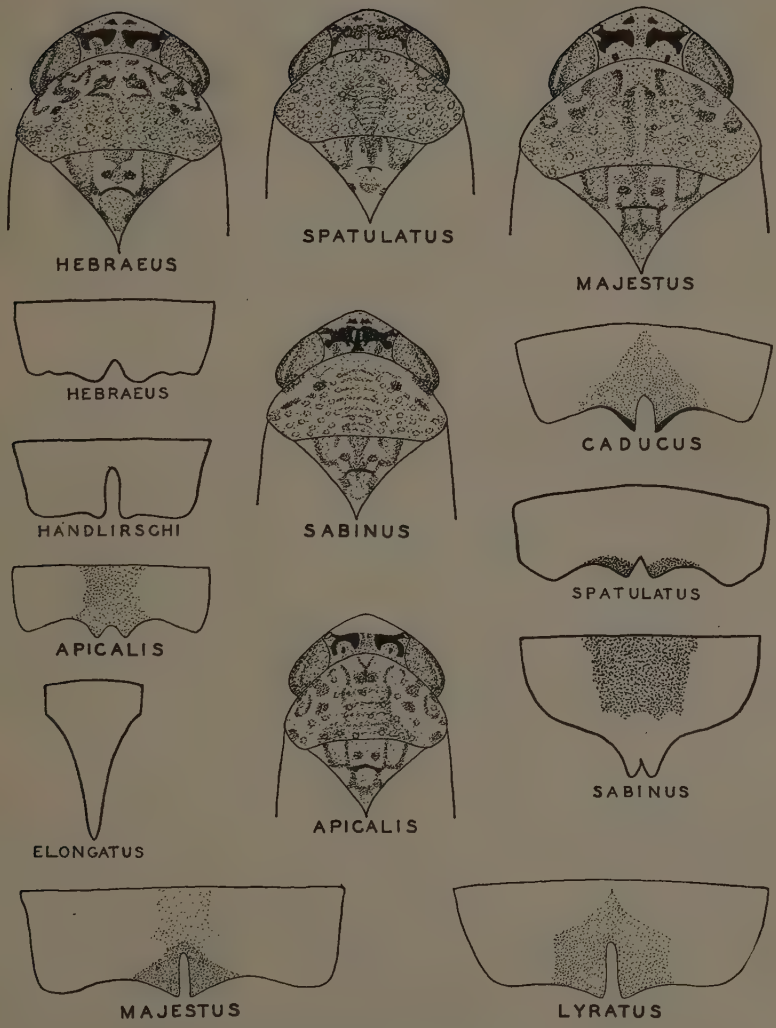
Phlepsius majestus Osborn and Ball. Proc. Ia. Acad. Sci. 4 : 229, 1897.

A large brownish species, resembling a member of the *Gyponinae* in general appearance. Length 9 to 10 mm.

The vertex is bluntly angled, about one-third longer on the middle than next the eyes, margin blunt. Vertex with a conspicuous, irregular, transverse fuscous band on the middle and usually two fuscous spots before it and above apex. Anterior portion of pronotum with dark vermiculate markings. Elytra heavily inscribed with chestnut brown pigment.

The female last ventral segment is three times as broad as long, the lateral angles broadly rounded. A narrow median incision half way to the base forms a rounded tooth on either side. The margin is broadly, shallowly emarginate either side between these and the lateral angles. Male plates rather long and narrow, apices broad, blunt and rounded. Aedeagus composed of a long curved, ventral process which is bifid at apex and a dorsal process which has a long, curved, tapered ventral portion and a shorter, broader dorsal portion, narrowed to a slender, apical finger process. Pygofer concavely rounded, dorsally and ventrally, and produced into a round pointed apex.

This species was described from specimens taken in Philadelphia, Pennsylvania, and has since been recorded for several of the eastern states. The types have been examined but specimens for all of the localities for which recorded have not been checked.



Heads and female genitalia of species of *Iowanus* as labeled.

Iowanus caducus (DeLong)

Texacanus caducus DeLong, Ohio Jour. Sci. 39 : 238, 1939.

Resembling *majestus* in form and coloration but with the apical lobe of the pygofer short, blunt, and not indented dorsally or ventrally at the base. Length male 8 mm.; female 9–9.5 mm.

The vertex is bluntly produced, more than half as long at middle as basal width between the eyes. The interrupted band between the eyes is interrupted at middle and enlarged at either end.

The female last ventral segment is scarcely concave between the lateral angles and a slightly produced blunt tooth either side of a rather narrow U-shaped notch reaching one-third the distance to the base. The male plates are long, tapered to rather narrow, rounded apices which exceed the pygofers in length. The pygofers with very short rounded caudal lobes which are not constricted or notched at base. The ventral portion of the aedeagus is long, apical fourth bent abruptly ventrally and cleft, forming two apical processes which are more slender than basal aedeagus body and are separated. The dorsal portion with a ventral, long, curved, slender process from the base of which arises a short, broader dorsal process.

This species is known to occur in Oklahoma, Tennessee and Illinois.

Iowanus borrori (DeLong)

Texananus borrori DeLong, Ohio Jour. Sci. 39 : 237, 1939.

Resembling *majestus* in form and coloration but different due to a bent terminal process of the aedeagus which is more than one-third as long as the basal portion and with a longer terminal lobe of the pygofer. Length 9.5 mm.

The vertex is quite broad and blunt, almost twice as wide between eyes at base as the median length. The dark trasnverse band between eyes is interrupted at the middle.

The female last ventral segment with prominent rounded lateral angles between which the posterior margin is concavely rounded either side of a rather long, sharp, pointed tooth which is produced on each side of a U-shaped notch which extends half way to the base of the segment. The male plates are long and slender, about as long as the pygofers which bear a long rather broad caudal lobe. The ventral portion of the aedeagus is short and robust. The apical third is bent abruptly ventrally, is cleft and forms two long apical widely separated spines. The dorsal portion consists of a long slender, curved ventral process from which arises an elongate, broader dorsal process at the base.

This species is known to occur in Ohio, Illinois and Minnesota.

Iowanus bullatus (DeLong)

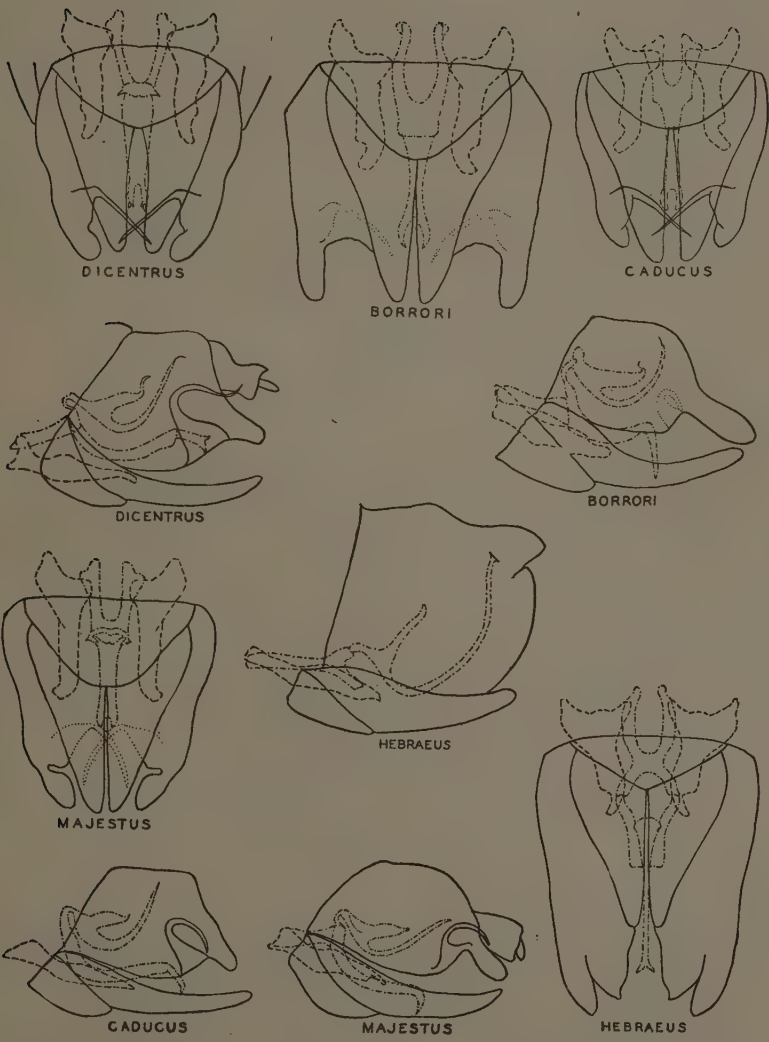
Texananus bullatus DeLong, Ohio Jour. Sci., 39 : 237, 1939.

Resembling *majestus* in form and coloration but distinguished from it by the broader, shorter, convexly rounded male plates and with a broadened basal lobe of aedeagus. Length male 9 mm.; female 10 mm.

Vertex broad, bluntly produced, length at middle more than half the basal width between the eyes. The vertex is marked with a pair of dark points above the apex and a broad straight interrupted dark band between the eyes.

The female last ventral segment is rather deeply broadly emarginate either side of a pair of approximate produced median teeth separated by a deep narrow incision extending half way to the base. The male plates are shorter than the pygofer and almost half as broad as long. The ventral portion of the aedeagus is cleft for a short distance at apex, forming a pair of blunt teeth which are slightly curved ventrally. The dorsal portion of the aedeagus with a long slender process curving caudally then dorsally from which arises a dorsal process at base which is rather broad, forming a rounded lobe. The caudal process of the pygofer is short and broad and broadly rounded.

This species is known from New Mexico only.



Ventral and lateral views of male genitalia of species of *Iowanus* in situ as labeled.

Iowanus dicentrus (DeLong)

Texananus dicentrus DeLong, Ohio Jour. Sci. 39 : 236, 1939.

In form, general appearance and coloration this species resembles *majestus*. Length male 8.5 mm.

Vertex bluntly angled, about three-fifths as long at middle as width between the eyes. The vertex is marked with a pair of brown spots just above apex. There is a transverse band between eyes interrupted at middle and forming a right triangular spot on either side, with the base of triangle along the median line.

The male plates are long, narrowed to bluntly rounded apices. The pygofers are shorter than the plates, the caudal lobe is elongate and narrow. The ventral portion of the aedeagus is forked for about one-fourth of its length at apex and each branch is conspicuously bifid at the apex, bearing a prominent dorsal and a ventral tooth. The dorsal portion of the aedeagus is composed of a long slender process which curves caudally then dorsally almost to the dorsal wall of the pygofer and a shorter dorsal process arising at the base and tapered to a blunt apex.

This species is known only by the type collected in Illinois. The aedeagus of the male is distinct.

Iowanus lyratus n. sp.

Resembling *majestus* in form and coloration but with male genitalia distinctive. Length 9–11 mm.

Vertex broad, scarcely angled, almost parallel margined, not quite twice as wide between eyes at base as median length.

Color of vertex pale, a pair of oblique pale brownish lines extending from beneath the ocelli across margin of vertex and terminating in a pair of proximal spots just above margin at apex. A broad black transverse band just back of ocelli interrupted at middle by a brownish longitudinal line. A pair of brownish dashes on base nearer each eye than middle. Pronotum with darker markings on anterior half. Elytra with ramose pigment lines and a few darker spots.

Female last ventral segment with posterior margin slightly emarginate between humeral angles and median produced portion which is narrowly incised nearly half way to the base. This incision forms a pair of approximate slightly produced, pointed teeth. The median third of segment embrowned. Male plates rather broad and short, bluntly pointed at apex. Styles rather broad at base, narrowed on apical two-thirds. Aedeagus with a ventral process which is long, slender and bifid for more than two-thirds its length; its two arms forming a lyre-shaped structure. The dorsal portion bears a basal process which is rather thick and shorter. The ventro-apical process is longer, slender, curved apically and dorsally.

Holotype male, Atlanta, Ga., September 14, 1939; allotype female same, August 27, 1939, and male paratype same locality, September 2, 1939; all collected by P. W. Fattig.

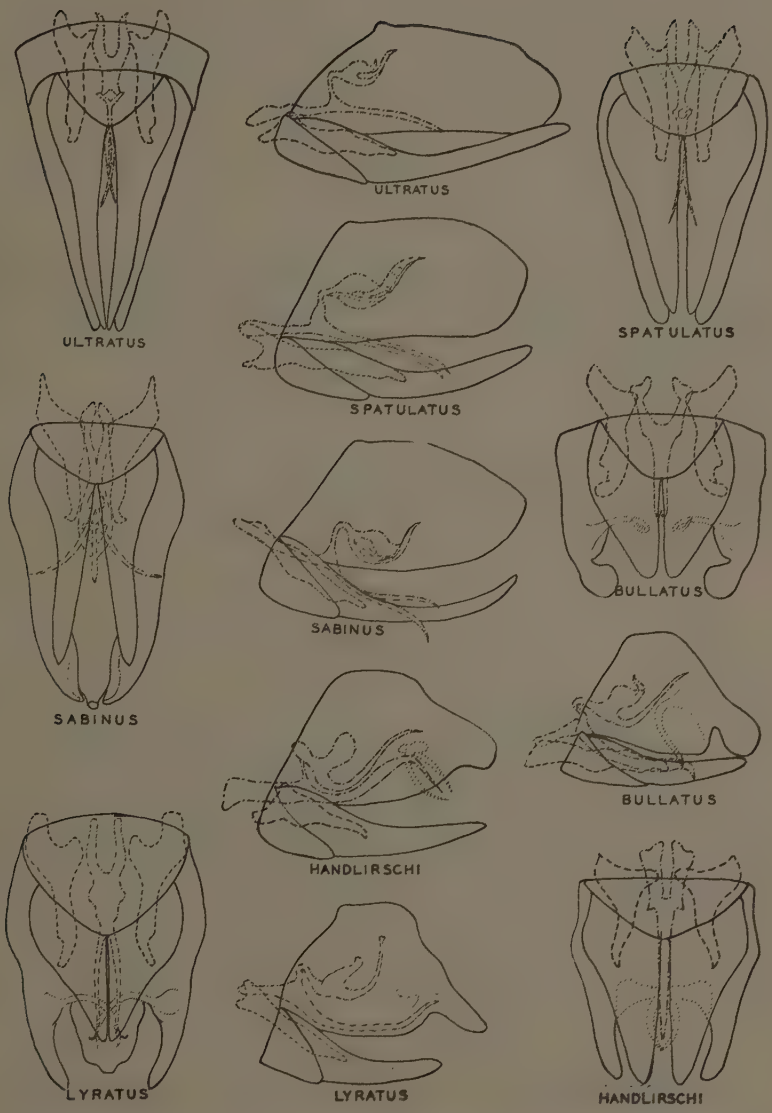
Iowanus handlirschi (Ball)

Phlepsius handlirschi Ball, Annals Ent. Soc. Amer. 11: 383, 1918.

Resembling *majestus* in form and coloration, but differing by having a different aedeagus, shorter blunter apical lobes on the pygofers and styles that are short, narrowed and sharply pointed at the apex. Length 9–10 mm.

Vertex blunt, transverse. The transverse dark band between the eyes is widened on the disc and interrupted in the middle. A pair of proximal spots are just above apex. Pronotum with coarse brown irrorations.

The female last ventral segment is almost truncate on posterior margin with a narrow U-shaped notch extending more than half way to the base. Male plates rather long, narrowed to bluntly pointed apices. Styles short, broad at base, rapidly narrowed at about half their length to form on the inner margin narrow, produced, sharply pointed apices. The aedeagus with a ventral and a dorsal process. The ventral portion is long, slender, curved dorsally, the



Ventral and lateral views of male genitalia of species of *Iowanus* in situ as labeled.

apical fourth bent ventrally and very narrow. The dorsal portion with a pair of dorsal, basal lobes from which a long, slender, tapering process extends ventrally then curves caudally and extends caudo-dorsally, and shorter than ventral portion. The apical lobes of pygofer short and broad, not constricted at base.

This species is known from Mexico only. It was originally described from Mexican material from Guerrero and has since been collected in other localities. The types have been examined and the illustrations made from Mexican material compared with the types.

Iowanus apicalis n. sp.

Resembling *handlirschi* in form and general appearance but with anterior half of vertex ivory white unmarked and with female genital segment more produced like *sabinus*. Length, female, 8 mm.

Vertex broad, bluntly angled, about one-half wider between eyes at base than median length.

Color pale with ramose pigment lines and dark spots or marks. Vertex with a broad black bar between the anterior margins of eyes broken at middle. The ends next the eyes narrower and split into divergent portions, one extending to ocellus and one directed posteriorly. Portion of vertex anterior to transverse bar, ivory white. Pronotum rather evenly mottled with dark brown. Scutellum with a few pale spots, elytra rather heavily marked with dark brown ramose pigment lines and spots.

Female last ventral segment almost truncate to median fifth which is produced and broadly shallowly notched at apex forming a pair of short pointed apical teeth.

Holotype female and paratype female collected at Mexcala, Gro., October 22, 1941 (K. 247) by Good and DeLong. A female specimen collected by Dr. Dampf at Tierra Blanca, Veracruz, July 29, 1932 (MF 2655) is also considered to belong to this species.

Iowanus sabinus (Sanders & DeLong)

Phlepsius sabinus Sanders and DeLong, Penna. Dept. Agri. Bur. Plant Ind. Bull. 3: 15, 1920.

Resembling *spatulus* in general form but more heavily marked with black and brown and with distinct genitalia. Length 6.5–7 mm.

Vertex bluntly, broadly angled, white anteriorly with two dark triangular spots just above apex. The transverse band between eyes is broad, irregular, extending to pronotum at middle and is black in color. The pronotum is pale, variously mottled with brown.

The female last ventral segment with posterior margin almost truncate to a median broad brown-tipped produced bifid tooth which is nearly one-third the length of the segment. Male plates long, rather narrow, bluntly pointed at apex. Styles tapered to form narrow apical half. Aedeagus with a long narrow bifid ventral portion and a dorsal portion which consists of three approximate processes. The ventral and median processes are long, narrow and curved. The dorsal process is broadened and shorter and lies within the concavity formed by the other processes.

Allotype male, Globe, Arizona, June 13, 1935, collected by F. H. Parker. This species was originally described from Arizona and has not been reported for other states.

Iowanus spatulatus (Van Duzee)

Phlepsius spatulatus Van Duzee, Trans. Amer. Ent. Soc. 19: 78, 1892.

This species is variously marked but without the definite black transverse bar between the eyes. Length 7–7.5 mm.

Vertex broad, bluntly produced, scarcely angled, more than one and one-half times as wide between eyes at base as median length.

Color pale, white to buff with dark brown markings. Vertex with a pair of approximate brown spots just above margin of vertex. An indefinite brownish transverse band between

the anterior margins of the eyes. Pronotum mottled with brown. Elytra pale with brown ramose pigment lines and spots.

Female last ventral segment with a distinct notch at middle of the posterior margin. This forms a blunt produced tooth each side of notch, between which and the humeral angle on each side the posterior margin is shallowly excavated. Male plates long, gradually tapered to acute apices. Styles broad at base, narrowed at about half their length and produced as narrow, almost parallel margined apices. The aedeagus has a pair of long slender ventral processes and a dorsal process composed of two parts—a ventral curved process and a shorter dorsal process.

This is a common and widely distributed species abundant in the herbaceous habitats in the southwestern U. S.

Iowanus ultratus (DeLong)

Texananus ultratus DeLong, Bull. Brook. Ent. Soc. 38: 126, 1943.

Resembling *spatulatus* in general form and appearance but with vertex more broadly rounded. The male plates are extremely long and narrow. Length, male, 7–8 mm.

The vertex is broadly and very bluntly angled, almost twice as wide between the eyes at base as median length. In color the vertex is pale yellow with the irregular brown irrorations forming a broken transverse band between the eyes. The pronotum is rather uniformly irrorate with brown.

The male plates are distinctly narrowed at one-third the distance from base, and produced as long narrow apical processes with pointed tips. Plates more than twice as long as combined basal width. The styles are long and narrow with blunt apices. The ventral portion of aedeagus with a pair of long slender apical processes which extend about one-half the length of the plates. The dorsal portion shorter with a ventral elongate process and a dorsal broader and shorter process.

This species is known from Arizona and Arkansas only.

Iowanus hebraeus (Ball)

Phlepsius hebraeus Ball, Annals Ent. Soc. Amer. 11: 383, 1918.

A large, rather distinctly marked species resembling *majestus* in general appearance. Length 8.5–9 mm.

Vertex about twice as wide as long, a little longer on middle than next the eyes. Vertex with the pair of small spots above apex and the dark transverse band interrupted at middle. Pronotum with several dark spots along anterior margin.

The female last ventral segment with a posterior margin which is slightly produced either side of a short broad median notch. Male styles narrow, decidedly shorter than pygofers, gradually tapered to bluntly pointed apices. Styles short, gradually narrowed to form short apical finger-like apices. Aedeagus long, slender, slightly bifid at apex. A short narrow process extends dorsally near base.

The male structures are quite different in type from those of the *majestus handlirschi* group..

This species was described from Mexico and has not been found in the United States.

A CHROMOSONE FREQUENCY TEST FOR LINKAGE OF THE RHESUS BLOOD FACTORS

DAVID C. RIFE,
The Ohio State University,
Columbus, Ohio

Two different hypotheses have been proposed to account for the inheritance of the Rhesus blood antigens. Wiener (3) explains their inheritance on the basis of a set of multiple alleles, while Fisher (2) accounts for it on the basis of three closely linked pairs of factors. At least six different genes are required for either explanation, as three different kinds of Rhesus antigen are known, each of which is dominant in its absence, but none of which show dominance to each other. If multiple alleles are responsible for the inheritance, it means that two genes ($Rh^{\circ I}$ and $Rh^{\circ II}$) each produce two different antigens. Linkage would mean that each gene would produce only one antigen. The genes and their products, both for multiple alleles and for linked genes are shown below, the three antigens being designated as antigen Rh°, antigen Rh', and antigen Rh''.

Multiple alleles	*Products*	*Linked genes*
Rh–	no antigen	c, d, e
Rh°	antigen°	D
Rh'	antigen'	C
Rh''	antigen''	E
$Rh^{\circ I}$	antigens°'	C, D
$Rh^{\circ II}$	antigens°''	D, E

Wiener (3) has tested the multiple allelic hypothesis with gene frequency analysis, and his results show close agreement between the expected and the actual frequencies. Fisher's hypothesis cannot be tested by the usual methods of gene frequency analysis. It is possible, however, to test the linkage hypothesis by *chromosome* frequency analysis, if we have data on the frequencies of the Rh types in a population in which mating has occurred at random. Within such populations, non-allelic genes should occur quite independently of each other. Even genes from sets of alleles too closely linked for any crossing over to occur should still show the same ratios of various combinations within chromosomes as expected on random assortment. That is to say, if alleles Aa and Bb are linked, regardless of how closely, and if the gene frequencies are A—.9; a—.1; B—.1; b—.9; we should expect the following chromosome ratios in the population:

A	B	.09
A	b	.81
a	B	.01
a	b	.09

The frequency of a chromosome carrying any combination of genes, then is the product of the frequencies of these genes.

This note is concerned with such a test for linkage of the Rh genes. Data on actual frequencies is based upon a population of 1,000 in New York City, tested by Wiener and Sonn (3). Gene frequencies are radily obtained from such data. For example, the frequency of gene c is the square root of the frequency of persons lacking antigen Rh'. It follows that $C=1-c$. In a similar manner the frequencies of genes D, d, E, and e may be readily ascertained.

C −.466
c −.534

D −.625
d −.375

E −.161
e −.839

The frequencies of the eight combinations of the Rhesus genes within chromosomes are easily computed and are shown below:

p	C	D	E	.047
q	C	D	e	.244
r	C	d	E	.028
s	c	D	E	.054
t	C	d	e	.147
u	c	d	E	.032
v	c	D	e	.280
w	c	d	e	.168

If p, q, r, s, t, u, v, and w represent the chromosome frequencies, the expected chromosome combinations within individuals are $(p+q+r+s+t+u+v+w)^2$. The frequencies of the expected Rh types within the population are as follows:

$$CDE = p^2+2pq+2pr+2ps+2pt+2pu+2pv+2pw+2qr+2qs+2qu+2rs+2st+2rv$$
$$CDe = q^2+2qt+2qv+2qw+2tv$$
$$cDE = s^2+2su+2sv+2sw+2uv$$
$$CdE = r^2+2rt+2ru+2rw+2tu$$
$$Dde = t^2+2tw$$
$$cdE = u^2+2uw$$
$$cDe = v^2+2vw$$
$$cde = w^2$$

The calculated and observed frequencies are shown below:

Classes	*Observed*	*Calculated*
CDE	164	181.963
CDe	541	432.216
CdE	0	29.624
cDE	128	72.686
Cde	9	71.001
cdE	3	11.776
cDe	26	172.480
cde	129	28.224
Total	1000	999.970

Extreme deviations of the observed from the expected are readily apparent, and indicate that the genes responsible for the inheritance of the Rh antigens are not linked.

LITERATURE CITED

(1) **Castle, W. B., M. W. Wintrobe, and L. H. Snyder.** 1948. On the nomenclature of the Anti Rh Typing Serums, Science 107: 27–31.
(2) **Fisher, R. A.** 1947. The Rhesus factor. American Scientist 35: 92–102, 113.
(3) **Wiener, A. S., and E. B. Sonn.** 1946. Annals of the New York Academy of Sciences XLVI: 883–992.

A STUDY OF THE RATE OF GROWTH OF TWO SCLEROTIZED REGIONS WITHIN LARVAE OF FOUR SPECIES OF MOSQUITOES

A. ABDEL-MALEK AND R. L. GOULDING, JR.[1]

Department of Zoology and Entomology,
The Ohio State University,
Columbus, Ohio

The observations leading to the following study were made independently by the joint authors and arose from two unconnected studies of mosquito larvae in which measurements of the larvae were a part of the routine. Upon discussion and comparison of data it was decided that, in spite of a difference in approach and method, the information should be pooled in order that a more complete study might be presented.

It is the objective of this paper to determine whether the measurements of head capsule width and air siphon length in different instars are so distinctly separated as to allow one to ascertain from these measurements the stadia or number of larval instars present.

MATERIALS AND METHODS

The larvae of *Aedes aegypti* L., *Ae. trivittatus* (Coq.), *Culex apicalis* Adams and *Anopheles quadrimaculatus* Say were measured for head capsule width and, with the exception of *An. quadrimaculatus*, for air siphon length. The larvae of *Ae. aegypti* and *Ae. trivittatus* were killed in K. A. A. D. killing mixture (Peterson, 1943). They were measured under a binocular microscope in which an ocular micrometer was inserted in one of the 15× oculars. In all the first instar larvae examined the objective used was 2× under which the ocular micrometer was calibrated to 0.09 mm per small division. The remaining instars were examined under a 1× objective with the micrometer calibrated to 0.045 mm. The larvae of both species were obtained from reared material, those of *Ae. trivittatus* from the eggs of wild, gravid females and those of *Ae. aegypti* from a culture maintained in the Department of Zoology and Entomology at the Ohio State University.

The larvae of *Culex apicalis* and *Anopheles quadrimaculatus* were killed in a solution containing six parts water, three parts ninety-five percent alcohol and one part formalin. They, too, were measured under a binocular microscope; however, in this case a 10× micrometer ocular was used with the opposite ocular blacked out. An objective of 48 mm focal length was employed in all measurements. The micrometer ocular was calibrated to 0.0216 mm per small division on the scale. The specimens of *C. apicalis* were taken from a field collection of larvae found in a road side permanent pool. Since several previous and succeeding collections from this pool yielded other species of *Culex* only rarely it was considered that all the instars were a satisfactory representation of the single species. The larvae of *An. quadrimaculatus* were taken from a culture maintained in the same laboratory with that of *Ae. aegypti*.

[1]The authors extend appreciation to Dr. E. L. Green, Dr. Alvah Peterson and Dr. Carl E. Venard, all of the Department of Zoology and Entomology at The Ohio State University, for their helpful suggestions and criticisms during the development of this study and preparation of the manuscript. Appreciation is also extended to the Statistics Laboratory of The Ohio State University for their statistical analysis of the data presented herein.

TABLE I

Aedes aegypti

INSTAR 1

Air Siphon	Head Capsule Width				Total
	202	225	247	270	
180	1	4	0	0	5
202	0	4	1	1	6
225	0	3	2	1	6
247	0	0	1	4	5
270	0	0	5	5	10
Total	1	11	9	11	32

Mean head capsule width: $h = 246$
Standard deviation of head capsule width: $s_h = 20.6$
Mean air siphon length: $a = 231$
Standard deviation of air siphon length: $s_a = 33.4$
Correlation coefficient between head capsule width and air siphon length: $r = .70$

INSTAR 2

Air Siphon	Head Capsule Width				Total
	360	405	450	495	
360	7	15	1	0	23
405	3	14	3	1	21
450	0	3	1	1	5
Total	10	32	5	2	49

$h = 402$
$s_h = 28.5$
$a = 388$
$s_a = 30.3$
$r = .36$

INSTAR 3

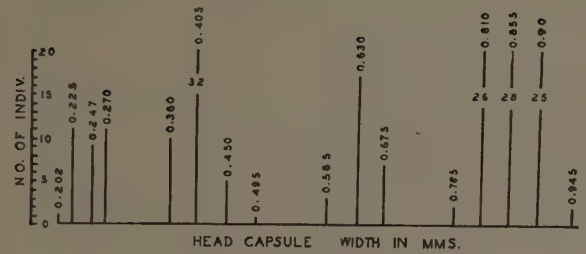
Air Siphon	Head Capsule Width			Total
	585	630	675	
540	0	3	0	3
585	3	9	1	13
630	1	6	6	13
Total	4	18	7	29

$h = 635$
$s_h = 27.8$
$a = 601$
$s_a = 30.1$
$r = .34$

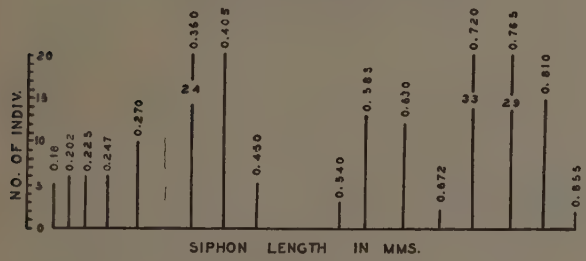
INSTAR 4

Air Siphon	Head Capsule Width					Total
	765	810	855	900	945	
675	0	1	0	1	0	2
720	1	18	11	3	0	33
765	1	7	11	11	0	30
810	0	0	5	9	1	15
855	0	0	1	1	1	3
Total	2	26	28	25	2	83

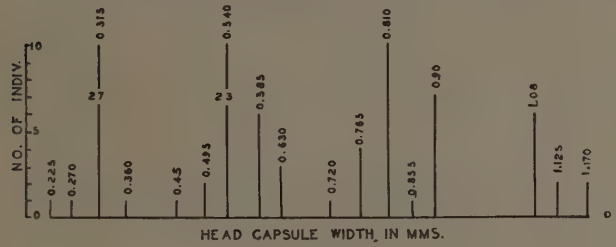
$h = 854$
$s_h = 40.7$
$a = 756$
$s_a = 40.1$
$r = .51$



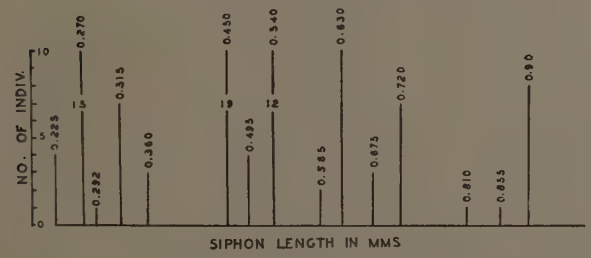
1. AEDES AEGYPTI



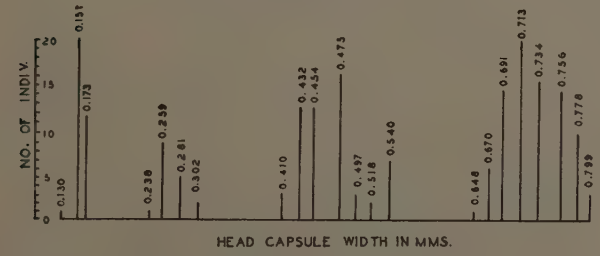
2. AEDES AEGYPTI



3. AEDES TRIVITTATUS



4. AEDES TRIVITTATUS



5. ANOPHELES QUAD.

FIGS. 1–5. Histograms of the head capsule widths and air siphon lengths of *Aedes aegypti*, *Aedes trivittatus* and *Anopheles Quad.*

TABLE II

Aedes trivittatus

INSTAR 1

Air Siphon	Head Capsule Width				Total	
	225	270	315	360		
225	1	0	3	0	4	h =312
270	0	1	14	0	15	s_h = 20.2
292	0	0	1	0	1	a =284
315	0	0	7	0	7	s_a = 37.6
360	0	0	2	1	3	r = .42
Total	1	1	27	1	30	

INSTAR 2

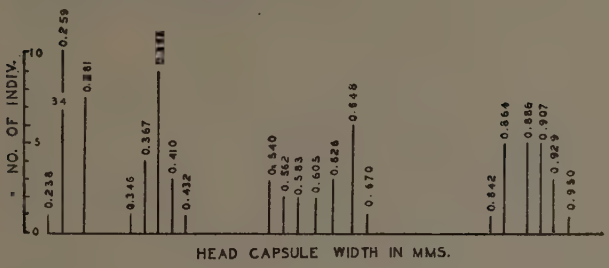
Air Siphon	Head Capsule Width					Total	
	450	495	540	585	630		
450	1	2	14	4	0	21	h =550
495	0	0	1	1	0	2	s_h = 36.3
540	0	0	8	1	3	12	a =483
Total	1	2	23	6	3	35	s_a = 42.7
							r = .57

INSTAR 3

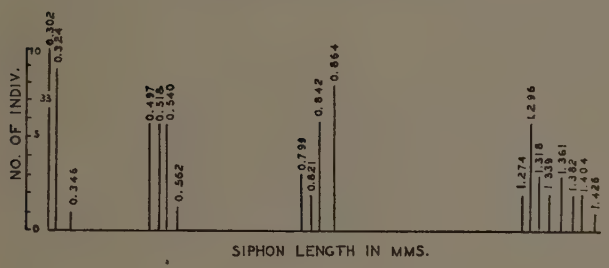
Air Siphon	Head Capsule Width					Total	
	720	765	810	855	900		
585	0	1	1	0	0	2	h =828
630	1	1	6	0	2	10	s_h = 55.5
675	0	1	0	0	2	3	a =667
720	0	1	3	0	3	7	s_a = 55.4
810	0	0	0	1	0	1	r = .32
Total	1	4	10	1	7	23	

INSTAR 4

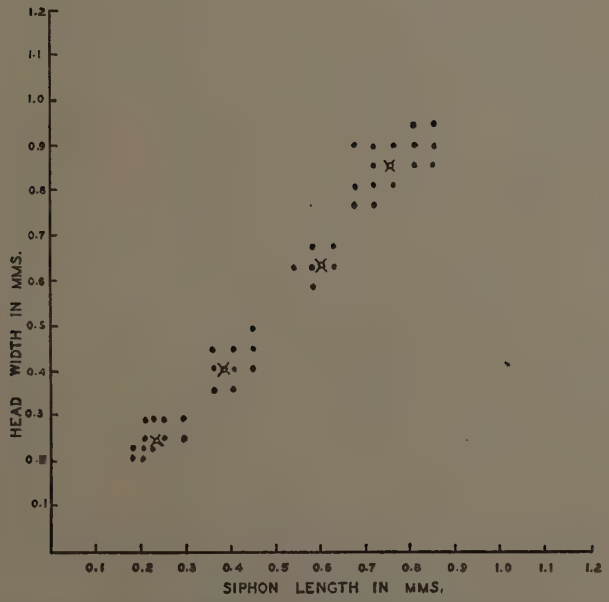
Air Siphon	Head Capsule Width			Total	
	1080	1125	1170		
810	1	0	0	1	h =1107
855	1	0	0	1	s_h = 37.9
900	4	2	2	8	a = 887
Total	6	2	2	10	s_a = 30.4
					r = .35



6. CULEX APICALIS



7. CULEX APICALIS



8. AEDES AEGYPTI

FIGS. 6–7. Histograms of the head capsule widths of air aiphon lengths of *Culex apicalia.*
FIG. 8. Scatter diagram of *Aedes aegypti.*

TABLE III

Culex apicalis

INSTAR 1

Air Siphon	Head Capsule Width			Total	
	238	259	281		
302	1	32	0	33	h =262.7
324	0	2	7	9	s_h = 9.33
346	0	0	1	1	a =307.9
Total	1	34	8	43	s_a = 10.6
					r = .81

INSTAR 2

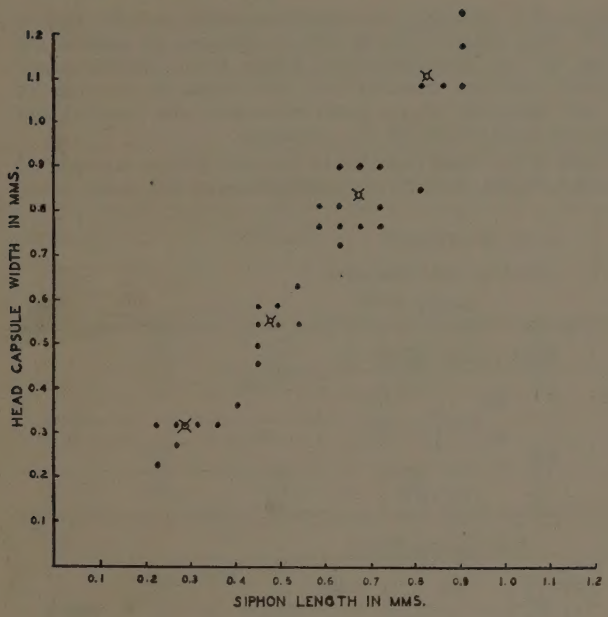
Air Siphon	Head Capsule Width					Total	
	346	367	388	410	432		
497	0	2	4	0	0	6	h =387.6
518	0	2	1	2	0	5	s_h = 20.2
540	1	0	4	1	0	6	a =520.8
562	0	0	0	0	1	1	s_a = 20.8
Total	1	4	9	3	1	18	r = .33

INSTAR 3

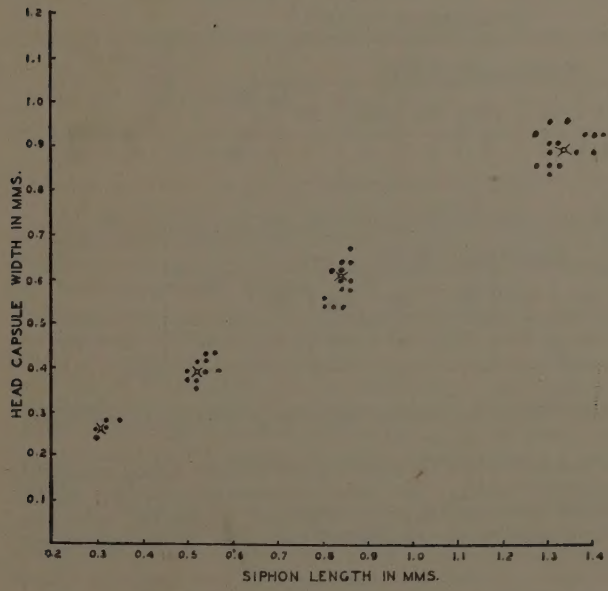
Air Siphon	Head Capsule Width							Total	
	540	562	583	605	626	648	670		
799	1	2	0	0	0	0	0	3	h =610.2
821	1	0	0	0	1	0	0	2	s_h = 27.7
842	1	0	1	1	2	1	0	6	a =843.5
864	0	0	1	1	0	6	1	9	s_a =237
Total	3	2	2	2	3	7	1	20	r =237

INSTAR 4

Air Siphon	Head Capsule Width						Total	
	842	864	886	907	929	950		
1274	0	2	0	0	0	0	2	h = 893.8
1296	1	2	1	2	0	0	6	s_h = 27.7
1318	0	1	0	2	0	0	3	a =1335.1
1339	0	0	0	1	0	1	2	s_a = 45.6
1361	0	0	3	0	0	0	3	r = .56
1382	0	0	0	1	1	0	2	
1404	0	0	1	0	1	0	2	
1426	0	0	0	0	1	0	1	
Total	1	5	5	6	3	1	21	



9. AEDES TRIVITTATUS



10. CULEX APICALIS

FIGS. 9–10. Scatter diagrams of *Aedes trivittatus* and *Culex apicalis*.

In the case of the larvae of *C. apicalis*, *An. quadrimaculatus* and *Ae. aegypti* the instars of each species were mixed together and withdrawn at random for measurement. The larvae of *Ae. trivittatus* were taken from predetermined instar groups. One hundred and two examinations were made in the case of *C. apicalis*, one hundred and ninety-six of *An. quadrimaculatus*, one hundred and ninety-two of *Ae. aegypti* and ninety-eight of *Ae. trivittatus*.

Measurements were made of the head capsules of the four groups represented in the region of greatest width, which occurred commonly between the ocular areas.

TABLE IV

Anopheles quadrimaculatus

INSTAR 1

Head Capsule Width			Total	
130	151	173		
1	21	12	34	$h = 158.2$ $s_h = 11.5$

INSTAR 2

Head Capsule Width				Total	
237	259	281	302		
1	9	5	2	17	$h = 269.4$ $s_h = 17.3$

INSTAR 3

Head Capsule Width							Total	
410	432	454	475	497	518	540		
3	13	13	17	3	2	7	58	$h = 467.8$ $s_h = 36.1$

INSTAR 4

Head Capsule Width								Total	
648	670	691	713	734	756	778	799		
1	6	15	21	16	15	10	3	87	$h = 727.2$ $s_h = 34.8$

The air siphons were measured from the point of juncture at the eighth abdominal segment to their tips.

Tables I-IV comprise a presentation of the head capsule and air siphon measurements in the form of frequency tables. The measurements are rounded off to three places and recorded in thousandths of a millimeter. Together with the frequency table will be found the means, standard deviation of the two variables and correlation coefficient.*

*The tables and statistical analysis included herein were prepared by the Statistical Laboratory, Ohio State University, Columbus, Ohio.

From the data in Tables I–IV, Table V has been compiled. The ratios refer to the ratio between the weighted mean of one instar and that of the preceding one. Difference refers to the differences between the weighted means.

TABLE V

	Head Capsule Width			Air Siphon Length		
Instar	Mean	Difference	Ratio	Mean	Difference	Ratio
			Aedes aegypti			
1	246			231		
2	402	156	1.634	388	157	1.680
3	635	233	1.580	601	213	1.549
4	854	219	1.345	756	155	1.258
			Aedes trivittatus			
1	312			284		
2	550	238	1.763	483	199	1.701
3	828	278	1.505	667	184	1.381
4	1107	279	1.337	887	220	1.330
			Culex apicalis			
1	263			308		
2	388	125	1.475	521	213	1.692
3	610	222	1.572	844	323	1.620
4	894	284	1.466	1335	491	1.582
			Anopheles quadrimaculatus			
1	158					
2	269	111	1.703			
3	468	199	1.740			
4	727	259	1.553			

DISCUSSION OF DATA

The distribution of the measurements of both the head capsule widths and air siphon lengths were plotted from the data in Tables I–IV. In addition, scatter diagrams were made by plotting the head capsule widths against the siphon lengths for *Aedes aegypti*, *Ae. trivittatus* and *Culex apicalis*.

Upon examination of the histograms (Plates I and II, Fig. 1–7) of both the head capsule widths and air siphon lengths it becomes apparent that these measurements are relatively constant within the four stadia. This observation corresponds with that of Dyar (1890) who, in his study of lepidopterous larvae, found in those examined that the sclerotized parts did not change in area during a stadium but rather the change occurred with ecdysis. In all cases, except the air siphons of the third and fourth instars of *Aedes aegypti*, there is a marked difference between the extremes.

It is considered that the accompanying scatter diagrams (Plates II and III, Fig. 8–10) serve to sum up the entire situation rather adequately. When length of siphon is plotted against head width for all individuals in each instar, it becomes readily apparent that both increase in a definite, clear-cut manner with ecdysis and that no overlapping of extremes occurs except in the case of the air siphons of the third and fourth instars of *Aedes trivittatus*.

Gaines and Campbell (1935) in their study of the number of instars in the corn earworm, *Heliothis obsoleta* (Fab.), used the ratio of the means of measurements of successive instars as a means of judging the progression of head capsule widths. They found, as did Ripley (1930) and Forbes (1934) with other lepidopterous larvae, that the greatest proportionate growth tended to fall between the first and second instars and the least between the pennultimate and the last. This also appears to hold true for the four groups of larvae considered here. An exception does occur in the head capsule ratios of *An. quadrimaculatus* and *C. apicalis* in which the ratios of the second and third instars are greater than those of the first and second instars; however, since the other five sets of ratios show a consistent decrease in ratio and since in no case is the last ratio greater than those preceding it, an experimental error rather than a deviation from the expected trend may be inferred.

The coefficients of correlation (r) indicate only a low degree of correlation between the size of the head capsules and air siphons. From this evidence it is not possible to predict size of an individual head capsule from the size of the air siphon or vice versa within a stadium.

Although there is an apparent and distinct increase in head capsule width and air siphon length between each instar and its succeeding one, it is not possible to state the nature of this increase in terms of regular progression, either geometric or arithmetic. This condition may be due to the fact that our time increment was determined arbitrarily and not with consideration of the time spent by each individual larva in each of the four stadia.

For practical purposes, the means of the head capsule widths of all the species studied and the air siphon lengths of the culicid species may be used as a criterion for determination of the instar. Although the character of the head capsule is known to be used by many field workers as an indication of instar, no stated corroboration of this practice was found in the literature in hand.

SUMMARY

The head capsule widths and air siphon lengths of the species of mosquito larvae herein examined are relatively constant within the four stadia. There was, with one exception, no overlapping of the extremes of measurements between instars.

The ratio between means of measurements of successive instars tends to decrease with the advancement of the larval instars.

Correlation is low between the size of head capsule and air siphon within an instar.

Determination of the nature of the rate of growth during successive molts cannot be completely assayed without an accurate determination of the time spent by each larva in each stadium.

For practical purposes the width of the head capsule and/or the length of the air siphon in each of the species considered may be used in determination of the stadia and number of instars.

BIBLIOGRAPHY

Dyar, H. G. 1890. The number of molts of Lepidopterous larvae. Psyche 5: 420–422.

Forbes, W. T. M. 1934. A note on Dyar's law (Lepidoptera larvae). Bull. Brooklyn Ent. Soc. 29: 146–149.

Gaines, J. C. and **F. L. Campbell.** 1935. Dyar's rule as related to the number of instars of the corn ear worm. *Heliothis obsoleta* (Fab.). Collected in the field. Ann. Ent. Soc. Amer. 28: 445–461.

Peterson, A. 1943. Some new killing fluids for larvae of insects. Jour. Ec. Ent. 36 (1): 151.

Ripley, L. B. 1923. The external morphology and postembryology of noctuid larvae. Ill. Biol. Monog. 8 (4): 102.